Phytochemical Screening of Syzygium caryophllatum and Syzygium nervosum (Myrtaceae)

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Abstract **- Objectives: The study aimed to identify the qualitative and quantitative phytochemical constituents present in the leaf, bark, root and seed of S. caryophllatum and S. nervosum (Myrtaceae) using the following solvents with different polarity: distilled water (aqueous), ethanol, ethyl acetate, methanol, acetone, chloroform and hexane.**

Methodology: The cleaned, healthy plant materials are cut into small sections and dried under shade for three to four weeks. The dried materials were ground into fine powder using electric grinder. Powder so obtained was stored in desiccators setup and used for extraction. Extraction was administered using 1gm of every sample coarsely powdered material with 25 ml of solvent and kept for 48 hrs with slight shaking. Here, distilled water (aqueous), ethanol, ethyl acetate, methanol, acetone, chloroform and hexane solvents were used for extraction.

Results: The study showed that the methanol, aqueous and ethanolic extracts contained most of the phytochemical constituents, followed by the ethyl acetate, hexane, acetone and chloroform respectively. These phytochemicals in the leaf, bark, root and seed include alkaloids; flavonoids; saponins; tannins; glycosides; phenols; proteins; terpenoids and steroid.

Conclusion: S. caryophllatum and S. nervosum leaf, stem, root and seed contain significant bioactive compounds that make the plant a potential antioxidant and among other therapeutic uses.

Index Terms **- S. caryophyllatum, S. nervosum, Phytochemical Screening.**

1.INTRODUCTION

The Plantae may be a storehouse of potential drugs and within the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less costly, safe, and efficient and infrequently have side effects. The plants which are selected for medicinal use over thousands of years constitute the foremost obvious choice of examining the present look for therapeutically effective new drugs such as anticancer drugs [1], antimicrobial drugs [2], antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the simplest source to get sort of drugs. About 80% of people from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to rose understand their properties, safety, and efficiency [3]. Medicinal plants contain some organic compounds which give definite physiological action on the physical body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4].

Syzygium is a genus of flowering plants belonging to the family Myrtaceae comprising of about 1200 species [5,6]. It is widely distributed spreads across in tropical Africa, sub-tropical and tropical Asia and Australia [7]. Several species such as S. jambos, S. aqueum and S. samarangense are grown and consumed for their edible fruits. Some are used in traditional medicine to treat inflammation, various allergic disorders, bronchitis, thirst, dysentery and ulcers [8]. Studies also revealed that extracts of different species in the genus Syzygium showed antibacterial, antifungal, antioxidant, anti-inflammatory, cytotoxic, antiHIV, antidiarrheal, anthelmintic, antinociceptive, antivirus and anticancer activities [9-19].

The medicinal value of plant lies within the phytochemical (bioactive) constituents of the plant which shows various physiological effects on physical body. Therefore, through phytochemical screening one could detect the various important compounds which may be used as the bases of modern drugs for curing various diseases [20]. Chemical compounds produced as a result of metabolic reaction during plant growth are known as phytochemicals. Harborne [21]

and Okwu [22] refer to such metabolic chemicals as "secondary metabolites" which include alkaloids, flavonoids, tannins, terpenes, terpenoids, phenols, gums, polysaccharides, and glycosides.

Plants of the genus syzygium are known as a rich source of secondary metabolites such as pentacyclic triterpenes and their glycoside derivatives, flavonoids, tannins and other aromatic compounds. Some of these secondary metabolites have been found to show antibacterial, antifungal, anticancer and hepatoprotective activities [23].

2. MATERIALS AND METHODS

2.1 Sample Collection:

Fresh and healthy plant parts of S. caryophyllatum and S. nervosum leaf, stem, seed and root were collected in a separate sterile polythene bag from Madurai, Tamil Nadu was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science, Plant Anatomy Research Centre, Tambaram. The register number of the specimen S. caryophyllatum (L.) Alston is PARC/2016/ 4448 and S. nervosum A. cunn. ex DC. is PARC/2016/4449.

2.2 Preparation of Solvent Extracts

The cleaned, healthy plant materials are cut in to small sections and dried under shade for three to four weeks. The dried material was ground into fine powder in an electric grinder. Powder so obtained was stored in desiccators setup and used for extraction. Extraction was carried out using 1gm of each sample coarsely powdered plant material with 25 ml of solvent and kept for 48 hrs with slight shaking. Here, aqueous, methanol, ethanol, ethyl acetate, hexane, acetone and chloroform solvents were used for extraction. The extraction was done at room temperature. All the extracts were filtered through Whatmann No.1 paper to get filtrate as extracts and were dried to concentrate the samples. The residual power was weighed and was re dissolved in the respective solvents to get a final concentration 1mg/ml. The powder was stored in airtight containers under refrigeration condition.

2.3 PHYTOCHEMICAL ANALYSIS

2.3.1QUALITATIVE ANALYSIS

2.3.1.1 Test for Tannins.

Analysis used was the method reported by Ejikeme et al. [24]. Each powder sample (0.30 g) was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 cm3 of water. Filtration was carried out after boiling using number 42 (125 mm)Whatman filter paper. To 5 cm3 of the filtrate was added 3 drops of 0.1% ferric chloride. A brownish green or a blue black colouration showed positive test.

2.3.1.2 Test for Saponin.

Methodology is as reported by Ejikeme et al. [24]. Distilled water (30 cm3) was added to powder samples (0.30 g) and boiled for 10 minutes in water bath and filtered using Whatman filter paper number 42 (125 mm). A mixture of distilled water (5 cm3) and filtrate (10 cm3) was agitated vigorously for a stable persistent froth. The formation of emulsion on addition of three drops of olive oil showed positive result.

2.3.1.3 Test for Steroid.

Analytical method used is according to Ejikeme et al. [24]. Each sample (0.30 g) weighed into a beaker was mixed with 20 cm3 of ethanol; the component was extracted for 2 hours. To the ethanolic extract of each sample (5 cm3) was added 2 cm3 acetic anhydride followed with 2 cm3 of concentrated tetraoxosulphate (VI) acid. A violet to blue or green colour change in sample(s) indicates the presence of steroids.

2.3.1.4 Test for Terpenoids.

Methodology is as reported by Ejikeme et al. [24]. Each powder sample (0.30 g) was weighed into a beaker and extracted with 30 cm3 and component extracted for 2 hours. A mixture of chloroform (2 cm3) and concentrated tetraoxosulphate (VI) acid (3 cm3) was added to 5 cm3 of each extract to form a layer. The presence of a reddish brown colouration at the interface shows positive results for the presence of terpenoids.

2.3.1.5 Test for Flavonoids.

The test for flavonoid adopted is as reported by Sofowara [25] and Harborne [26]. Each sample (0.30 g) weighed into a beaker was extracted with 30 cm3 of distilled water for 2 hours and filtered with Whatman filter paper number 42 (125 mm). To 10 cm3 of the aqueous filtrate of each extract was added 5 cm3 of 1.0 M dilute ammonia solution followed by the addition of 5 cm3 of concentrated tetraoxosulphate (VI) acid. Appearance of yellow colouration which disappeared on standing shows the presence of flavonoids.

2.3.1.6 Test for Alkaloids.

Test for alkaloids used is as reported by Hikino et al. [27]. Extraction of component from 2 grams of each powder sample was carried out using 5% tetraoxosulphate (VI) acid (H2SO4) (20 cm3) in 50% ethanol by boiling for 2 minutes and filtered through Whatman filter paper number 42 (125 mm). The filtrate was made alkaline using 5 cm3 of 28% ammonia solution (NH3) in a separating funnel. Equal volume of chloroform (5.0 cm3) was used in further solution extraction in which chloroform solution was extracted with two 5 cm3 portions of 1.0 M dilute tetraoxosulphate (VI) acid. This final acid extract was then used to carry out the following test: 0.5 cm3 of Dragendorff 's reagent (Bismuth potassium iodide solution) was mixed with 2 cm3 of acid extract and precipitated orange colour infers the presence of alkaloid.

2.3.1.7 Test for Glycoside.

Glycoside test was conducted according to the method reported by Hikino et al. [27]. To 2.00 g of each sample was added 20 cm3 of water, heated for 5 minutes on a water bath and filtered through Gem filter paper (12.5 cm). The following tests were carried out with the filtrate: (a) 0.2 cm3 of Fehling's solutions A and B was mixed with 5 cm3 of the filtrate until it became alkaline (tested with litmus paper). A brick-red colouration on heating showed a positive result. (b) Instead of water, 15 cm3 of 1.0 M sulphuric acid was used to repeat the above test and the quantity of precipitate obtained compared with that of (a) above. High precipitate content indicates the presence of glycoside while low content shows the absence of glycoside.

2.3.1.8 Test for Phenols

Phenols test was conducted according to the method reported by R. Suman Kumar et al. [28]. To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

2.3.1.9 Test for Resins

Resins test was conducted according to the method reported by R. Suman Kumar et al. [28]One ml of various solvent extract was treated with few drops of acetic anhydride solution followed by one ml of conc. H2SO4. Resins give colouration ranging from orange to yellow.

2.3.1.10 Test for Quinones

Quinones test were conducted according to the method reported by R. Suman Kumar et al. [28]. One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

2.3.1.11 Test for Phytosterols (Salkowski's Test)

Phytosterols test was conducted according to the method reported by Rajani Yadav et al. [29] The plant extract was mixed with chloroform and filtered. The filtrateis treated with 5-6 drops of conc. Sulphuric acid carefully and shaken gently, allowed to stand. A golden yellow colour indicates the presence of triterpens (phytosterol).

2.3.2 QUANTITATIVE DETERMINATION OF PRIMARY METABOLITES

2.3.2.1 Determination of carbohydrate

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0 ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30˚C for 20 min. The absorbance was read at 490 nm [30].

2.3.2.2 Determination of protein

The dried and powdered samples was extracted by stirring with 50 ml of 50% methanol (1:5 w/v) at 25 °C for 24 h and centrifuged at 7,000 rpm for 10 min .0.2 ml of extract was pipette out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed it to stand for 10 min. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660 nm [31].

2.3.2.3Estimation of total lipid content

10 gm sample was used to extract lipids with 150 ml of petroleum ether for 16 hr, at a solvent condensation rate of 2–3 drops/sec according to AACC Approved

Method 30-25 with minor modifications of sample size and extraction time. The obtained extract was concentrated and evaporated at room temperature to dryness. The weight of extract gives the total lipid content which was expressed as mg/g dry matter [32].

2.3.3 Quantitative Determination of Secondary Metabolites:

2.3.3.1 Estimation of Alkaloids

Alkaloids were determined using Harborne method [33]. Five grams of the sample was weighed into a 250 ml beaker, 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.3.3.2 Estimation of Flavonoids

The total flavonoid content in the sample was estimated by the method of Chang [34]. A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after six minutes 0.1 5 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm along with standard quercetin at 5 - 25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent / gm of dried sample.

2.3.3.3 Total Phenolic Content

Total phenolic content of extract was determined according to the Folin-Ciocalteau method of Slinkard and Singleton [35] with some modifications. Briefly, 0.1 ml of extract (200, 600 and 1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin-Ciocalteau's reagent were seeded in a tube, and then 1 ml of sodium carbonate was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

2.3.3.4 Total Tannins Content

Tannins - phenolics were determined by the method of Peri and Pompei [36]. 1 ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract.

2.3.3.5 Total Saponins

The extracts were ground and 20 g of extract put into a conical flask and 100 ml of 20% ethanol is added to the sample [37]. The sample is heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture is then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90 ºC. The concentrate is then transferred into a 250 ml separating funnel and 20 ml of diethyl ether is added to the extract and vigorously shaken. The aqueous layer is recovered while the diethyl ether layer is discarded and the purification process is repeated. 60 ml of n-butanol is added and the combined n-butanol extracts is washed twice with 10 ml of 5% sodium chloride. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight and values are expressed as mg/g of extract.

3. RESULTS

3.1 Qualitative phytochemical analysis

3.1.1Qualitative phytochemical analysis of leaf extract of S. caryophyllatum and S. nervosum

The qualitative phytochemical analysis was carried out in S. caryophyllatum leaf extract, with different solvents such as ethanol, ethyl acetate, methanol, hexane, acetone, chloroform and aqueous extracts are given in table 1.

In S. caryophyllatum, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin,

tannins, phenols, carbohydrates, proteins, glycosides, terpenoids , quinone and lipids and absence of steroids, resins and phytosterol in aquesous extract. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, carbohydrates, proteins and lipids and absence of glycosides, resins, phytosterol, terpenoids and quinone. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, glycosides, resins, quinone, carbohydrates, proteins and lipids and absence of only phytosterol, terpenoids. In ethyl acetate, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, steroids, protein, lipid, terpenoids and absence of glycosides, resins, phytosterol, carbohydrate and quinone. In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, phytosterol, carbohydrates, proteins and lipids and absence of steroids, glycosides, resins, terpenoids and quinone. In acetone, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, steroids, glycosides and absence of terpenoids, resins, phytosterol, carbohydrate, quinone, protein and lipid. In chloroform, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, and lipids and absence of carbohydrates, proteins, steroids, glycosides, resins, phytosterol, terpenoids , and quinine.

In aqueous extract of S. nervosum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins, glycosides, terpenoids , quinone and lipids and absence of steroids, resins and phytosterol. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, carbohydrates, proteins and lipids and absence of glycosides, resins, phytosterol, terpenoids and quinone. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, glycosides, resins, phytosterol, carbohydrates, proteins and lipids and absence of only quinone, terpenoids. In ethyl acetate, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, steroids, protein, lipid, terpenoids and absence of glycosides, resins, steroids, phytosterol, carbohydrate and quinone. In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, phytosterol, carbohydrates, proteins, lipids terpenoids and quinone and absence of steroids, glycosides and resins. In acetone, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, steroids, glycosides and absence of terpenoids, resins, phytosterol, carbohydrate, quinone, protein and lipid. In chloroform, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, proteins and lipids and absence of carbohydrates, glycosides, resins, phytosterol, terpenoids, and quinine.

3.1.2Qualitative phytochemical analysis of bark extract of S. caryophyllatum and S. nervosum

The qualitative phytochemical analysis was carried out in S. caryophyllatum bark extract, with different solvents such as ethanol, ethyl acetate, methanol, hexane, acetone, chloroform and aqueous extracts as shown in table 2.

The phytochemical analysis of S. caryophyllatum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, carbohydrates, proteins, glycosides, and lipids and absence of resins, phytosterol, terpenoids and quinone in aqueous extract. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, quinone, carbohydrates, proteins and lipids and absence of steroids, glycosides, resins, phytosterol, terpenoids. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, quinone, steroids, glycosides, terpenoids, carbohydrates, proteins and lipids and absence of only resins and phytosterol. In ethyl acetate, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, protein and quinone and absence of steroids, glycosides, resins, phytosterol, carbohydrates, lipid and terpenoids. In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins and lipids and absence of steroids, glycosides, phytosterol, terpenoids, resins and quinone. In acetone, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols,

steroids, carbohydrate, proteins, lipids and absence of glycosides, terpenoids, resins, phytosterol, and quinone. In chloroform, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, steroids, carbohydrate, proteins, lipids and absence of glycosides, terpenoids, resins, phytosterol, and quinone.

In aqueous, the phytochemical analysis of S. nervosum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, carbohydrates, proteins, glycosides, and lipids and absence of resins, phytosterol, terpenoids and quinone. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins and lipids and absence of steroids, glycosides, resins, phytosterol, terpenoids, quinone. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, quinone, steroids, glycosides, terpenoids, carbohydrates, proteins and lipids and absence of only resins and phytosterol. In ethyl acetate, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, proteins, carbohydrates and quinone and absence of steroids, glycosides, resins, phytosterol, lipid and terpenoids. In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins and lipids and absence of steroids, glycosides, phytosterol, terpenoids, resins and quinone. In acetone, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, steroids, carbohydrate, protein , lipid and absence of glycosides, terpenoids, resins, phytosterol, and quinone. In chloroform, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, carbohydrate, protein, lipid and absence of glycosides, terpenoids, resins, phytosterol, steroids and quinone.

3.1.3 Qualitative phytochemical analysis of root extract of S. caryophyllatum and S. nervosum

The qualitative phytochemical analysis was carried out in S. caryophyllatum root extract, with different solvents such as ethanol, ethyl acetate, methanol, hexane, acetone, chloroform and aqueous extracts were represented in table 3.

The phytochemical analysis of S. caryophyllatum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins, resins, phytosterol, terpenoids and lipids and absence of quinone, glycosides and steroids. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, glycosides, terpenoids, carbohydrates, proteins, phytosterol and lipids and absence of steroids, resins and quinine. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, glycosides, steroids, phytosterols, terpenoids, quinone, carbohydrates, proteins and lipids and absence of only resins. In ethyl acetate, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, glycosides, phytosterols, terpenoids, proteins and lipids and absence of resins, steroids, quinone and carbohydrates. In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, terpenoids, carbohydrates and lipids and glycosides, resins, phytosterols, proteins and quinone. In acetone, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, phytosterols, carbohydrates, proteins and lipids and absence of terpenoids, quinone, glycosides and resins. In chloroform, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, phytosterols, terpenoids and carbohydrates and absence of glycosides,resins, quinone, proteins and lipids.

In aqueous, the phytochemical analysis of S. nervosum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins, resins, phytosterol, terpenoids and lipids and absence of quinone, glycosides and steroids. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, glycosides, terpenoids, carbohydrates, proteins, phytosterol and lipids and absence of steroids, resins and quinine. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, glycosides, steroids, phytosterols, terpenoids, quinone, carbohydrates, proteins and lipids and absence of only resins. In ethyl acetate, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, glycosides, phytosterols, terpenoids, proteins and lipids and absence of resins, steroids, quinone and carbohydrates. In hexane, the phytochemical analysis

showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, terpenoids, carbohydrates, proteins and lipids and glycosides, resins, phytosterols and quinone.In acetone, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, phytosterols and absence of terpenoids, quinone, glycosides, resins, carbohydrates, proteins and lipids.In chloroform, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, steroids, phytosterols, terpenoids, proteins and carbohydrates and absence of glycosides,resins, quinone and lipids.

3.1.4 Qualitative phytochemical analysis of seed extract of S. caryophyllatum and S. nervosum

The qualitative phytochemical analysis was carried out in S. caryophyllatum seed extract, with different solvents such as ethanol, ethyl acetate, methanol, hexane, acetone, chloroform and aqueous extracts are given in table 4.

The phytochemical analysis of S. caryophyllatum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins, steroids and lipids and absence of glycosides, resins, phytosterols, terpenoids and quinone. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins, glycosides and lipids and absence of resins, phytosterols, terpenoids, steroids and quinone. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, quinone, resins, glycosides, phytosterol, carbohydrates, proteins and lipids and absence of only steroids and terpenoids. In ethyl acetate, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, ,resins, terpenoids, quinone, carbohydrates, proteins and lipids and absence of glycosides, steroids and phytosterols. In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates and lipids and absence of glycosides, resins, steroids, phytosterols, terpenoids, quinone and proteins. In acetone, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, resins , carbohydrates, proteins and lipids and absence of glycosides, steroids, phytosterols, terpenoids and quinone. In chloroform, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins and lipids and absence of glycosides, resins, steroids, phytosterols, terpenoids and quinone.

In aqueous, the phytochemical analysis of S. nervosum showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins, steroids and lipids and absence of glycosides, resins, phytosterols, terpenoids and quinone. In ethanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates, proteins and lipids and absence of resins, glycosides, steroids, phytosterols, terpenoids, steroids and quinone. In methanol, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, quinone, resins, glycosides, phytosterol, carbohydrates, proteins and lipids and absence of only steroids and terpenoids. In ethyl acetate, the phytochemical analysis showed positive response to alkaloids, flavonoids, tannins, saponin, phenols, ,resins, terpenoids, quinone, carbohydrates, proteins and lipids and absence of glycosides, steroids and phytosterols.

In hexane, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, carbohydrates and lipids and absence of glycosides, resins, steroids, phytosterols, terpenoids, quinone and proteins. In acetone, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, resins, carbohydrates, proteins and lipids and absence of glycosides, steroids, phytosterols, terpenoids, protein and quinone. In chloroform, the phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, tannins, phenols, phytosterols, resins, carbohydrates, proteins and lipids and absence of glycosides, steroids, terpenoids and quinone.

TABLE 1: Shows the qualitative phytochemical analysis of S. caryophyllatum and S. nervosum leaf extract

Phytochemicals	Aqueous		Ethanol		Methanol		Ethyl acetate		Hexane		Acetone		Chloroform	
	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	-	-	+	+	-	-	-	-	+	+	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Steroids	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Phytosterols	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Terpenoids	+	+	-	-	-	-	+	+	-	-	-	-	-	-
Quinone	+	+	-	-	+	-	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	-	-	+	+	-	-	-	-
Proteins	+	+	+	+	+	+	+	-	+	+	-	-	-	+
Lipids	+	+	+	+	+	+	+	+	+	+	-	-	+	+

+ = Presence, - = Absence, *S.cer= *S. caryophyllatum*, S.ner = *S. nervosum*

Table 2: Shows the qualitative phytochemical analysis of S. caryophyllatum and S. nervosum bark extract

Phytochemicals	Aqueous		Ethanol		Methanol		Ethyl acetate		Hexane		Acetone		Chloroform	
	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	-	-	+	+	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	-	-	+	+	+	+	-	-	+	+	+	-
Phytosterols	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Quinone	-	-	-	-	+	+	+	+	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipids	+	+	+	+	+	+	-	-	+	+	+	+	+	+

+ = Presence, - = Absence, *S.cer= *S. caryophyllatum*, S.ner = *S. nervosum*

Table 3: Shows the qualitative phytochemical analysis of S. caryophyllatum and S. nervosum root extract

Phytochemicals	Aqueous		Ethanol		Methanol		Ethyl acetate		Hexane		Acetone		Chloroform	
	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	-	-	+	+	+	+	+	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	+	+	-	-	+	+	+	+	+	+
Phytosterols	+	+	+	+	+	+	+	+	-	-	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Quinone	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	-	-	+	+	+	-	+	+
Proteins	+	+	+	+	+	+	+	+	-	+	+	+	-	+
Lipids	+	+	+	+	+	+	+	+	+	+	+	-	-	-

+ = Presence, - = Absence,S.cer= S. caryophyllatum, S.ner = S. nervosum***

Table 4: Shows the qualitative phytochemical analysis of S. caryophyllatum and S. nervosum seed extract

Phytochemicals	Aqueous		Ethanol		Methanol		Ethyl acetate		Hexane		Acetone		Chloroform	
	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner	S. car	S. ner
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	-	-	+	-	+	+	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	-	-	-	-	+	+	+	+	-	+	+	-	-	+
Steroids	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Phytosterols	-	-	-	-	+	+	-	-	-	-	-	-	+	+
Terpenoids	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Quinone	-	-	-	-	+	+	+	+	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	-	-	+	-	+	+
Lipids	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Presence, - = Absence, **S.cer= S. caryophyllatum, S.ner = S. nervosum*

3.2 Quantitative estimation of primary metabolites of S. caryophyllatum and S. nervosum leaf, bark, root and seed extracts Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds [38]. In the present investigation, primary metabolites like carbohydrates, protein and lipids were quantitatively analyzed as shown in table 5.

In S. caryophyllatum the higher amount of carbohydrates was present in the leaf extract (45.41 mg/g dw) followed by seed extract (43.12 mg/g dw) and minimal amount of carbohydrates was found in the bark extract (19.5 mg/g dw). In S. nervosum maximum amount of carbohydrates were present in leaf extract (52.82 mg/g dw) followed by seed extract (33.75 mg/g dw) and lower level of carbohydrates was found in the bark extract (28.11mg/g dw).In S. caryophyllatum the maximum yield of proteins was found in these seed extract (32.13 mg/g dw) followed by leaf extract (14.21 mg/g dw) and lower level of proteins was found in the bark extract (10.24 mg/g dw). In S. nervosum maximum amount of proteins were present in seed extract (39.83 mg/g dw) followed by leaf extract (21.96 mg/g dw) and minimal amount of proteins was found in the bark extract (16.14 mg/g dw).In S. caryophyllatum the higher amount of lipids was present in the seed extract (32.56 mg/g dw) followed by leaf extract (29.31 mg/g dw) and lower level of lipids was found in the bark extract (10.03 mg/g dw). In S. nervosum maximum amount of lipids were present in seed extract (33.62 mg/g dw) followed by leaf extract (31.29 mg/g dw) and lower level of lipids was found in the root extract (12.22 mg/g dw).

Table 5: Quantification of primary metabolites of S. caryophyllatum and S. nervosum leaf, bark, root and seed extracts

S.NO	Primary Metabolites	Weight (mg/g dw)							
		Leaf extract		Bark extract		Root extract		Seed extract	
		S.car	S.ner	S.car	S.ner	S.car	S.ner	S.car	S.ner
1	Carbohydrates	45.41	52.82	19.5	28.11	32.15	28.45	43.12	33.75
2	Proteins	14.21	21.96	10.24	16.14	13.46	18.41	32.13	39.83
3	Lipids	29.31	31.29	10.03	13.92	14.88	12.22	32.56	33.62

*S.cer= S. caryophyllatum, S.ner = S. nervosum

3.3 Quantitative estimation of secondary metabolites of S. caryophyllatum and S. nervosum leaf, bark, root and seedextracts

Local inhabitant knowledge and literature about the curative properties helped for the selection of the plants under study. Detection of alkaloids, flavonoids, saponins, tannins and phenols in several extracts indicates that these were major secondary metabolites.

3.3.1 Estimation of Alkaloids

In S. caryophyllatum, the results showed (table 6 – 13) that the content of total alkaloids in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the alkaloids content is ranged from 23.13 to 54.16 mg/g dw. The highest yield of alkaloids content was present in methanol extract (54.16 mg/g dw) followed by aqueous extracts (51.32 mg/g dw) and lowest amount were present in acetone extracts (23.13mg/g dw). In bark extracts the alkaloids content is ranged from 16.58 to 50.34 mg/g dw. The highest yield of alkaloids content was present in methanol extract (50.34 mg/g dw) followed by ethyl acetate extract (44.16 mg/g dw) and lowest amount were present in chloroform extract (16.58 mg/g dw). In root extracts the alkaloids content were ranged from 10.12 to 48.34 mg/g dw. The maximum amount was present in ethanol extract (48.34 mg/g dw) followed by aqueous extract (45.12mg/g dw) and minimal amount was present in acetone extract (10.12mg/g dw). In seed extracts the shows the alkaloids content ranged from 19.48 to 48.8 mg/g dw. The higher yield was found in ethanol extract (48.8mg/g dw), followed by aqueous extract (47.01mg/g dw) and minimum in acetone extract (19.48 mg/g dw). Among all extract the maximum amount was found in methanol extract of S.caryophyllatum leaf and minimum in acetone extract of S.caryophyllatum root extract.

In S. nervosum, the results showed (table 6 – 13) that the content of total alkaloids in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the alkaloids content is ranged from 29.12 to 59.32 mg/g dw. The highest yield of alkaloids content was present in methanol extract (59.32 mg/g dw) followed by ethyl acetate extracts (47.39 mg/g dw) and lowest amount were present in acetone extracts (29.12mg/g dw). In bark extracts the alkaloids content is ranged from 24.39 to 46.32 mg/g dw. The highest yield of alkaloids content was present in ethanol extract (46.32 mg/g dw) followed by methanol extract (46.08 mg/g dw) and lowest amount were present in chloroform extract (24.39mg/g dw). In root extracts the alkaloids content were ranged from 17.48 to 45.02 mg/g dw. The

maximum amount was present in ethanol extract (45.02 mg/g dw) followed by methanol extract (43.24 mg/g dw) and minimal amount was present in acetone extract (17.48 mg/g dw). In seed extracts the shows the alkaloids content ranged from 23.61 to 46.18 mg/g dw. The higher yield was found in methanol extract (46.18 mg/g dw), followed by ethanol extract (45.24 mg/g dw) and minimum in hexane extract (23.61 mg/g dw). Among all extract the maximum amount was found in methanol extract of S.nervosum leaf and minimum in acetone extracts of S.nervosum root extract.

3.3.2 Estimation of flavonoid

Flavonoids are important because of their ability to inhibit enzymes, anti-inflammatory activity and antimicrobial activity. In S. caryophyllatum, the results showed (table 6 – 13) that the content of total flavonoids in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the flavonoids content is ranged from 14.15 to 63.43 mg/g dw. The highest yield of flavonoids content was present in methanol extract (63.43 mg/g dw) followed by ethanol extracts (57.45 mg/g dw) and lowest amount were present in chloroform extracts (14.15mg/g dw). In bark extracts the flavonoids content is ranged from 30.21 to 58.81 mg/g dw. The highest yield of flavonoids content was present in methanol extract (58.81 mg/g dw) followed by aqueous extract (48.07 mg/g dw) and lowest amount were present in chloroform extract (30.21 mg/g dw). In root extracts the flavonoids content were ranged from 13.24 to 46.83 mg/g dw. The maximum amount was present in ethyl acetate extract (46.83 mg/g dw) followed by ethanol extract (42.54mg/g dw) and minimal amount was present in chloroform extract (13.24 mg/g dw). In seed extracts the shows the flavonoids content ranged from 21.48 to 47.12 mg/g dw. The higher yield was found in methanol extract (47.12mg/g dw), followed by ethanol extract (35.67mg/g dw) and minimum in chloroform extract (21.48 mg/g dw). Among all extract the maximum amount was found in methanol extract of S.caryophyllatum leaf and minimum in chloroform extract of S.caryophyllatum root extract.

In S. nervosum, the results showed (table 6 – 13) that the content of total flavonoids in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the flavonoids content is ranged from 29.43 to 59.25 mg/g dw. The highest yield of flavonoids content was present in ethanol extract (59.25 mg/g dw) followed by methanol extracts (52.42 mg/g dw) and lowest amount were present in chloroform extracts (29.43mg/g dw). In bark extracts the flavonoids content is ranged from 18.56 to 53.01 mg/g dw. The highest yield of flavonoids content was present in ethyl acetate extract (53.01 mg/g dw) followed by aqueous extract (49.1 mg/g dw) and lowest amount were present in acetone (18.56 mg/g dw). In root extracts the flavonoids content were ranged from 32.19 to 51.16 mg/g dw. The maximum amount was present in ethanol extract (51.16 mg/g dw) followed by aqueous extract (50.25mg/g dw) and minimal amount was present in acetone extract (32.19 mg/g dw). In seed extracts the shows the flavonoids content ranged from 20.19 to 51.31 mg/g dw. The higher yield was found in methanol extract (51.31mg/g dw), followed by ethanol extract (48.66 mg/g dw) and minimum in acetone extract (20.19 mg/g dw). Among all extract the maximum amount was found in ethanol extract of S.nervosum leaf and minimum in S.nervosum bark, acetone extract.

3.3.3 Total saponins content (TPC)

Saponins are reported to have hypocholesterolemic and antidiabetic properties, while triterpenoids display analgesic and anticancer properties (Ali et al., 2008). So these secondary metabolites contribute to potent use of plants in pharmacological industries. In S. caryophyllatum, the results showed (table 6 – 13) that the content of total saponins in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the saponins content is ranged from 22.1 to 68.22 mg/g dw. The highest yield of saponins content was present in ethanol extract (68.22 mg/g dw) followed by aqueous extracts (64.05 mg/g dw) and lowest amount were present in acetone extracts (22.1 mg/g dw). In bark extracts the saponins content is ranged from 24.31 to 43.16 mg/g dw. The highest yield of saponins content was present in ethyl extract (43.16 mg/g dw) followed by ethanol extract (42.38 mg/g dw) and lowest amount were present in hexane extract (24.31 mg/g dw). In root extracts the saponins content were ranged from 12.02 to 58.07 mg/g dw. The maximum amount was present in ethanol extract (58.07 mg/g dw) followed by aqueous extract (49.11mg/g dw) and minimal amount was present in acetone extract (12.02mg/g dw). In seed extracts the shows the saponins content ranged from 18.52 to 62.42 mg/g dw. The higher yield was found in ethanol extract

(62.42mg/g dw), followed by methanol extract (60.32mg/g dw) and minimum in acetone extract (18.52 mg/g dw). Among all extract the maximum amount was found in ethanol extract of S.caryophyllatum leaf and minimum in acetone extract of S.caryophyllatum root extract.

In S. nervosum, the results showed (table 6 – 13) that the content of total saponins in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the saponins content is ranged from 23.19 to 79.48 mg/g dw. The highest yield of saponins content was present in ethanol extract (79.48 mg/g dw) followed by methanol extracts (75.64 mg/g dw) and lowest amount were present in chloroform extracts (23.19mg/g dw). In bark extracts the saponins content is ranged from 19.42 to 59.58 mg/g dw. The highest yield of saponins content was present in ethanol extract (59.58 mg/g dw) followed by aqueous extract (56.43 mg/g dw) and lowest amount were present in chloroform extract (19.42 mg/g dw). In root extracts the saponins content were ranged from 18.61 to 59.46 mg/g dw. The maximum amount was present in aqueous extract (59.46 mg/g dw) followed by methanol extract (58.45mg/g dw) and minimal amount was present in chloroform extract (18.61 mg/g dw). In seed extracts the shows the saponins content ranged from 36.12 to 53.31 mg/g dw. The higher yield was found in aqueous extract (53.31mg/g dw), followed by methanol extract (49.68 mg/g dw) and minimum in acetone extract (36.12 mg/g dw). Among all extract the maximum amount was found in ethanol extract of S.nervosum leaf and minimum in chloroform extracts of S.nervosum root extract.

3.3.4 Total content of tannins

In S. caryophyllatum, the results showed (table 6 – 13) that the content of total tannins in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the tannins content is ranged from 12.34 to 52.36 mg/g dw. The highest yield of tannins content was present in methanol extract (52.36 mg/g dw) followed by ethanol extracts (45.67 mg/g dw) and lowest amount were present in chloroform extracts (12.34mg/g dw). In bark extracts the tannins content is ranged from 30.31 to 48.19 mg/g dw. The highest yield of tannins content was present in methanol extract (48.19 mg/g dw) followed by ethanol extract (45.41mg/g dw) and lowest amount were present in hexane extract (30.31 mg/g dw). In root extracts the tannins content were ranged from 11.04 to 33.67 mg/g dw. The maximum amount was present in methanol extract (33.67 mg/g dw) followed by hexane extract (29.14mg/g dw) and minimal amount was present in acetone extract (11.04mg/g dw). In seed extracts the shows the tannins content ranged from 22.21 to 55.71 mg/g dw. The higher yield was found in methanol extract (55.71mg/g dw), followed by aqueous extract (39.57mg/g dw) and minimum in chloroform extract (22.21 mg/g dw). Among all extract the maximum amount was found in methanol extract of S.caryophyllatum seed and minimum in acetone extract of S.caryophyllatum root extract.

In S. nervosum, the results showed (table 6 – 13) that the content of total tannins in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the phenols content is ranged from 22.56 to 57.02 mg/g dw. The highest yield of tannins content was present in aqueous extract (57.02 mg/g dw) followed by methanol extracts (51.62 mg/g dw) and lowest amount were present in hexane extracts (22.56mg/g dw). In bark extracts the tannins content is ranged from 21.36 to 59.14 mg/g dw. The highest yield of tannins content was present in methanol extract (59.14 mg/g dw) followed by ethyl acetate extract (42.17 mg/g dw) and lowest amount were present in ethanol extract (21.36 mg/g dw). In root extracts the tannins content were ranged from 23.44 to 53.03 mg/g dw. The maximum amount was present in ethanol extract (53.03 mg/g dw) followed by aqueous extract (52.05mg/g dw) and minimal amount was present in acetone extract (23.44 mg/g dw). In seed extracts the shows the tannins content ranged from 36.92 to 51.64 mg/g dw. The higher yield was found in methanol extract (51.64mg/g dw), followed by aqueous extract (49.13 mg/g dw) and minimum in chloroform extract (36.92 mg/g dw). Among all extract the maximum amount was found in methanol extract of S.nervosum bark and minimum in ethanol extracts of S.nervosum bark extract.

3.3.5. Total phenols content (TPC)

In S. caryophyllatum, the results showed (table 6 – 13) that the content of total phenols in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the phenols content is ranged from 15.17 to 49.81 mg/g dw. The highest yield of phenols content was present in methanol extract (49.81 mg/g dw) followed by ethanol extracts (46.92 mg/g dw) and lowest

amount were present in chloroform extracts (15.17mg/g dw). In bark extracts the phenols content is ranged from 15.86 to 39.22 mg/g dw. The highest yield of phenols content was present in methanol extract (39.22 mg/g dw) followed by chloroform extract (28.11 mg/g dw) and lowest amount were present in hexane (15.86mg/g dw). In root extracts the phenols content were ranged from 14.67 to 44.12 mg/g dw. The maximum amount was present in methanol extract (44.12 mg/g dw) followed by acetone extract (36.45mg/g dw) and minimal amount was present in hexane extract (14.67mg/g dw). In seed extracts the shows the phenols content ranged from 22.42 to 46.28 mg/g dw. The higher yield was found in ethanol extract (46.28mg/g dw), followed by methanol extract (33.46mg/g dw) and minimum in acetone extract (22.42 mg/g dw). Among all extract the maximum amount was found in ethanol extract of S.caryophyllatum seed and minimum in hexane extract of S.caryophyllatum root.

In S. nervosum, the results showed (table 6 – 13) that the content of total phenols in leaf, bark, root and seed extracts, varied to a great extent. In leaf extracts the phenols content is ranged from 21.35 to 57.14 mg/g dw. The highest yield of phenols content was present in ethanol extract (57.14 mg/g dw) followed by methanol extracts (55.41 mg/g dw) and lowest amount were present in ethyl acetate extracts (21.35mg/g dw). In bark extracts the phenols content is ranged from 12.12 to 50.41 mg/g dw. The highest yield of phenols content was present in methanol extract (50.41 mg/g dw) followed by ethyl acetate extract (45.19 mg/g dw) and lowest amount were present in chloroform extract (12.12 mg/g dw). In root extracts the phenols content were ranged from 29.73 to 39.88 mg/g dw. The maximum amount was present in methanol extract (39.88 mg/g dw) followed by aqueous extract (39.45mg/g dw) and minimal amount was present in chloroform extract (29.72 mg/g dw). In seed extracts the shows the phenols content ranged from 18.17 to 46.31 mg/g dw. The higher yield was found in ethanol extract (46.31mg/g dw), followed by methanol extract (45.83 mg/g dw) and minimum in acetone extract (18.17 mg/g dw). Among all extract the maximum amount was found in ethanol extract of S.nervosum leaf and minimum in chloroform extract of S.nervosum bark extract.

Table 6: Shows the quantitative phytochemical activity of secondary metabolites of *S. caryophyllatum* leaf extract

Phytochemical constituents (mg/g)	Concentration in mg/g of LEAF EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	23.13	51.32	24.16	43.21	38.06	33.42	54.16
Flavonoids	17.82	41.28	14.15	57.45	35.51	35.72	63.43
Saponins	36.18	64.05	22.1	68.22	54.19	40.7	62.55
Tannins	25.68	42.13	12.34	45.67	33.49	23.42	52.36
Phenols	21.56	17.53	15.17	46.92	24.19	30.12	49.81

Table7: Shows the phytochemical activity of secondary metabolites of *S. caryophyllatum* bark extract

Phytochemical constituents (mg/g)	Concentration in mg/g of BARK EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	26.28	40.56	16.58	46.55	44.16	28.15	50.34
Flavonoids	34.18	48.07	30.21	43.21	46.32	41.07	58.81
Saponins	25.22	40.12	27.56	42.38	43.16	24.31	35.16
Tannins	32.21	41.13	31.08	45.41	32.08	30.31	48.19
Phenols	24.53	28.12	28.21	33.78	24.67	15.86	39.22

Table 8: Shows the phytochemical activity of secondary metabolites of *S. caryophyllatum* root extract

Phytochemical constituents (mg/g)	Concentration in mg/g of ROOT EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	10.12	45.12	11.28	48.34	21.06	16.31	35.91
Flavonoids	20.32	42.34	13.24	42.54	46.83	28.01	32.92
Saponins	12.02	49.11	23.65	58.07	45.21	16.32	23.68
Tannins	11.04	26.18	18.61	19.25	23.1	29.14	33.67
Phenols	36.45	29.42	29.48	25.03	32.06	14.67	44.12

Table 9: Shows the phytochemical activity of secondary metabolites of *S. caryophyllatum* seed extract

Phytochemical constituents (mg/g)	Concentration in mg/g of SEED EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	19.48	47.01	27.51	48.8	32.1	28.45	44.52
Flavonoids	25.53	23.71	21.48	35.67	33.12	21.56	47.12
Saponins	18.52	42.11	33.14	62.42	21.76	23.48	60.32
Tannins	30.59	39.57	22.21	31.55	36.32	33.82	55.71
Phenols	22.42	23.28	25.23	46.28	23.41	32.52	33.46

Table 10: Shows the phytochemical activity of secondary metabolites of *S. nervosum* leaf extract

Phytochemical constituents (mg/g)	Concentration in mg/g of LEAF EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	29.12	36.54	30.77	45.51	47.39	39.36	59.32
Flavonoids	33.09	29.48	29.43	59.25	45.9	48.36	52.42
Saponins	38.56	52.46	23.19	79.48	51.55	26.08	75.64
Tannins	31.06	57.02	26.83	48.06	34.56	22.56	51.62
Phenols	36.51	29.44	26.21	57.14	21.35	27.46	55.41

Table 11: Shows the phytochemical activity of secondary metabolites of *S. nervosum* bark extract

Phytochemical constituents (mg/g)	Concentration in mg/g of BARK EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	25.68	39.01	24.39	46.32	39.42	30.48	46.08
Flavonoids	18.56	49.1	39.16	47.08	53.01	38.66	48.21
Saponins	29.63	56.43	19.42	59.58	54.19	23.12	46.86
Tannins	26.88	34.65	29.19	21.36	42.17	37.43	59.14
Phenols	16.91	39.64	12.12	39.16	45.19	42.31	50.41

Table 12: Shows the phytochemical activity of secondary metabolites of *S. nervosum* root extract

Phytochemical constituents (mg/g)	Concentration in mg/g of ROOT EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	17.48	39.48	25.87	45.02	34.12	27.34	43.24
Flavonoids	32.19	50.25	42.17	51.16	39.11	49.6	48.61
Saponins	20.34	59.46	18.61	54.75	54.01	43.48	58.45
Tannins	23.44	32.05	29.89	53.03	48.22	49.06	49.64
Phenols	30.64	39.45	29.73	34.18	34.12	32.19	39.88

TABLE 13: Shows the phytochemical activity of secondary metabolites of *S. nervosum* seed extract

Phytochemical constituents (mg/g)	Concentration in mg/g of SEED EXTRACT						
	Acetone	Aqueous	Chloroform	Ethanol	Ethyl acetate	Hexane	Methanol
Alkaloids	30.02	38.31	27.33	45.24	45.11	23.61	46.18
Flavonoids	20.19	45.31	28.64	48.66	39.88	23.48	51.31
Saponins	39.44	53.31	49.68	47.68	36.12	47.14	39.56
Tannins	49.13	46.32	36.92	47.31	40.33	47.21	51.64
Phenols	18.17	40.56	32.78	46.31	43.49	39.88	45.83

4.DISCUSSION

Phytochemical screenings bring forth essential information about the medicinal importance of the plants. Phenolic compound plays an important role in plant defence mechanisms against invading microorganisms and other types of environmental stress, such as wounding and excessive light or ultraviolet radiation. These compounds can be treated as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system. Flavonoids, tannins and other polyphenol derivatives are found to be higher in many species of Syzygium. In accordance with previous report, our results showed that high phenol content in S. caryophllatum leaf methanol extracts may be the reason for its high anti-glycation activity. Flavonoids, tannins, anthocyanins and other phenolic compounds present in bark, leaves and fruits are act as natural antioxidants. In alloxan-induced diabetic rats, damaged pancreatic cell can be regenerated with the help of naturally occurring flavonoids. Tannins were reported to exhibit antibacterial antiviral, and anti-tumour activities. Some kinds of tannins can helps to lower the activity of mutagenicity of a number of mutagens and also exhibited antioxidant, antimicrobial and anticarcinogenic activities. Seeds of S. cumini one of the close related species of S.caryophyllatum which processes high flavonoid contents, which account for the scavenging of free radicals and a protective effect on antioxidant enzymes. Presence of phenolic compounds in each plant is varying with its environments. For example, Archana et a., [39] have reported 0.089-0.096 mg gallic acid equivalent/mg plant material of total phenolic content in S. cumini fruit extract. Total phenolic content of 299.64 and 782.86 mg gallic acid equivalent/mg plant material of S. jombo bark extract have been reported by Islam et al,. [40]

Our investigation of in vitro studies of the parts (leaf, bark, root and seed) methanol extracts of S.nervosum also showed remarkably high alkaloid, flavonoids, tannins, saponins and phenols content when compared with S. caryophyllatum. In S. caryophyllatum, alkaloids, flavonoids and phenols were found to be maximum in methonolic extract of leaf. While, saponins are found to be maximum in ethonolic extract of leaf; tannins were showed maximum in methonolic extract of seed. In S. nervosum, alkaloids were found to be maximum in methonolic extract of leaf; ethonolic extract of leaf showed maximum yield of flavonoids, saponins and phenols; tannins were found

to be maximum in methonolic extract of bark. In our studies, of the parts (leaf, bark, root and seed) leaf extract of S.caryophyllatum and S.nervosum showed maximum amount of phytochemical constituents.

5. CONCLUSION

Our result shows the presence of medicinally important constituents in the plants studied. Phytochemical contribute medicinal as well as physiological properties to the plants in the treatment of different ailments. Therefore, extracts from these plants might be seen as an honest source for useful drugs. The traditional medicine practice is usually recommended strongly for these plants also because it is suggested that further work should be administered to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Further works elucidate the possible mechanism of action of these extracts. Phytochemical analysis of S. caryophyllatum and S. nervosum (leaf, bark, root and seed) extracts showed that, they contain alkaloids, flavonoids, saponins, tannins and phenols, but the alkaloids, flavonoids and saponins were found in greater amount. In our studies, methonolic leaf extract of S.nervosum showed maximum amount of phytochemical constituents than S.caryophyllatum.

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