Extraction Phytochemical Screening and Anti-inflammatory Activity of Balanitis Ingudi

AKSHAY WASKAR[1], DR. BRIJESH SIROHI[2], DR. SHILENDRA LARIYA[3]
[1, 2, 3] *Radharaman College of Pharmacy, Bhopal (M.P)*

Abstract*— The Aim of this study is to evaluate the phytochemical components and anti-inflammatory activity of Balanitis ingudi. Balanitis ingudi is a small shrub native to India and is used in traditional Ayurvedic medicine. The plant has been used to treat a variety of ailments such as skin diseases, ulcers, burns, coughs, sore throats, and more. The plant is reported to have anti-inflammatory, antispasmodic, and diuretic properties, which can help to reduce inflammation and improve the overall health of the body. In evaluation of anti-inflammatory activity, the carrageenan-induced paw edema model in rats was employed. Different doses of the Balanites aegyptiaca extract were administered, and the results demonstrated a dose-dependent reduction in paw edema. Group II, treated with 100 mg/kg, exhibited a notable decrease, while Group III, treated with 200 mg/kg, showed a further reduction, suggesting a potential dose-dependent effect. The positive control, Group IV, treated with Indomethacin, displayed a significant reduction in paw edema, validating the anti-inflammatory potential of the extract.

***Index Terms*— Inflammation, Balanitis Ingudi, Paw edema, Herbal medicine**

I. INTRODUCTION

The body's normal and necessary response to signals from tissue injury or pathogenic infection is inflammation (Kotas et al., 2015). Inflammation after injury or infection promotes the return of homeostasis by defending the host against foreign pathogens and by healing injured tissue (Rock et al., 2009). A pro-inflammatory response is normally triggered quickly during the induction phase of inflammation, which is progressively followed by a resolution phase (Schett and Neurath, 2018). Inflammation is therefore essential for wound healing. The formation and progression of numerous inflammatory disorders, including as cancer, obesity, sepsis, cardiovascular, neurological, and autoimmune diseases, can occur if this carefully planned response is dysregulated, however (Furman et al., 2019).

Chronic or local inflammation that is out of control happens in stages. When pathogens or injured cells send warning signals like damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), local immune cells are activated, producing cytokines, chemokines, proteases, growth factors, and oxygen-free radicals (Zindel and Kubes, 2020). Through endothelial cell adhesion, circulating immune cells subsequently target the area of the injured tissue to increase pro-inflammatory responses (Luster et al., 2005).

Homeostasis is ultimately restored by this well-coordinated immunological response. Late-stage inflammation, however, can occur if the reaction is weak or overwhelming, disturbing the harmony between the innate and adaptive arms of the immune system. Such a dysregulated immune response can set off a catastrophic cascade that is characterized by excessive generation of danger signals and local or systemic tissue damage. A breakdown in immunological tolerance and the advancement of inflammatory disease, which may potentially be fatal, can come from changes in the inflammatory response from acute to chronic (Rajendran et al., 2018). Understanding of the relationship between inflammation and homeostasis is growing, and a holistic approach to the inflammatory process presents prospects for the development of targeted and effective treatment approaches to control inflammation (Darnell and Mooney, 2017). Inflammation is frequently treated with medication therapy, and biomaterials can be employed as drug carriers to permit regulated drug release with great efficacy and few side effects. Biomaterials can also scavenge pro-inflammatory chemicals or prevent undesirable leukocyte-endothelial cell interactions to decrease inflammation in addition to drug delivery8. Thus, by providing design flexibility, route targeting, and great spatiotemporal control of anti-inflammatory

effects, biomaterials can supplement conventional pharmacological therapy.

II. THE INFLAMMATORY ENVIRONMENT

- Danger signals -

PAMPs released by bacteria and viruses trigger inflammation in infection; DAMPs are the endogenous counterpart of PAMPs, inducing sterile inflammation (that is, inflammation owing to trauma rather than infection) (Tang et al., 2012). DAMPs and PAMPs are recognized by different innate immune pattern recognition receptors, such as Toll-like receptors (TLRs), which are expressed on immune cells (Tang et al., 2012., Cen et al., 2018). Upon binding extracellular DAMPs and PAMPs, TLRs activate cytoplasmic adapter molecules that initiate a cascade of activation pathways, including nuclear factor-κB (NF-κB), interferon regulatory factor and a link to MAPK pathways, leading to the production of pro-inflammatory cytokines (for example, tumour necrosis factor (TNF), interleukin-1 (IL-1) and IL-6) and chemokines through transcriptional and post-transcriptional mechanisms (Tang et al., 2012., Cen et al., 2018). Furthermore, reactive oxygen and nitrogen species (RONS) can activate IκB kinases and/or inhibit phosphor tyrosine and phosphoserine/threonine phosphatases to upregulate redox-sensitive NF-κB, further exacerbating inflammation (El-Kenawi and Ruffell, 2017). In addition to TLRs, immune cells possess NOD-like receptors (NLRs) to specifically identify pathogenic patterns in the cytoplasm, resulting in an inflammasome-mediated activation of pro-inflammatory cytokine release. An auxiliary mechanism in dealing with inflammation involves cyclic GMP–AMP synthase and its downstream effector, stimulator of interferon genes, which recognizes intracellular DNAs and promotes the release of type I interferons and other inflammatory cytokines (Barber et al., 2015). Therefore, immune cells can recognize and respond to various danger signals in the initial stage of inflammation and transmit signals to the nucleus for the production of cytokines.

- Inflammatory Cells

Innate immune cells, that is, monocytes, macrophages and neutrophils, orchestrate early inflammation (Kurts and Schwesinger, 2019., Neurath, 2019). Within minutes of an injury, tissue-resident macrophages and circulating neutrophils are activated by DAMPs and release inflammatory mediators that recruit circulating innate immune cells to the injury site (McDonald and Kubes, 2016). Additional mechanosignalling is triggered by direct interaction between surface molecules in adjacent cells (Gargalionis et al., 2018, Knapik et al., 2014). In response to macrophage-produced cytokines and PAMPs or DAMPs, released by injured or invading cells, the postcapillary venular endothelium upregulates adhesion molecules involved in the leukocyte recruitment cascade (Kreuger and Phillipson, 2016, Kolaczkowska, E. and Kubes,2013). This cascade involves mechanosignalling between adhesion molecules on the endothelium and various integrins on leukocytes, which can direct the recruitment of circulating leukocytes, eventually leading to their extravasation. Neutrophils are the first immune cells to arrive at the affected site, and intravascular neutrophils adhered to the endothelium can modify the endothelial barrier (Nemeth et al., 2020). In response to chemoattractants, neutrophils release TNF in close proximity to endothelial junctions to locally increase microvascular permeability (Finsterbusch et al., 2014). Circulating neutrophils migrate to the site as early as 20 min after injury and accumulate for the next 2 h. Circulating monocytes subsequently infiltrate the site, 24 h after injury, and increase in number for up to 72 h post-injury (Baggiolini, 1998). These infiltrating monocytes produce inflammatory mediators, clear dead cells, stimulate extracellular matrix production and angiogenesis, and regenerate parenchymal cells. In early inflammation, CD4+ T helper 1 (TH1) cells infiltrate the injury site and release pro-inflammatory cytokines (Baggiolini, 1998., Sallusto and Baggiolini, 2008). In late inflammation, regulatory T (Treg) cells and TH2 cells, along with regulatory M2-like macrophages, increase in number and resolve inflammation by producing anti-inflammatory cytokines, such as transforming growth factor-β (TGFβ) and IL-10 (Larouche et al., 2014).

- Inflammatory mediators

Activated inflammatory cells release acute mediators of inflammation (Atri et al., 2018), such as the pro-inflammatory cytokines TNF, IL-1β and IL-6, which have profound effects on tissue regeneration, response to infection or pain, and neuronal activity (Back et al.,

2019, Mantovani et al., 2019). When dysregulated, these proinflammatory cytokines can contribute to the pathogenesis of diseases, such as systemic inflammatory response syndrome, atherosclerosis, rheumatoid arthritis, multiple sclerosis and septic shock (McInnes et al., 2016).

RONS, including oxygen and nitrogen free radicals, such as superoxide radical (O2 •−), hydroxyl radical (• OH) and nitric oxide radical (• NO), are pro-inflammatory molecules that cause lipid peroxidation and oxidative stress (Yang et al., 2019, Tejera et al., 2019). RONS can have deleterious effects on tissues and are, thus, regulated by endogenous antioxidant mechanisms that involve enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) and antioxidants (ascorbic acid, α-tocopherol and glutathione). Overproduction of RONS results in oxidative stress and tissue damage.

Enzymes play essential roles in inflammation and wound healing (Duffin et al., 2016, Ramachandran et al., 2016). Cyclooxygenase 1 and cyclooxygenase 2 are upregulated in leukocytes and metabolize arachidonic acid into prostaglandins (PGs) (for example, PGE2, PGD2, PGF2), which regulate vascular permeability, neuron activity, bronchial reactivity and cardiovascular smooth muscles (Daniel et al., 2019). Activated inflammatory cells produce proteolytic enzymes that activate the complement, kallikrein–kinin and coagulation cascades, as well as proteinase-activated receptors, integrins and other adhesion receptors and ion channels, triggering the five cardinal signs of inflammation: increased blood flow and redness, fluid leakage and swelling, pain, heat and loss of tissue function.

Neutrophil extracellular traps (NETs) are large molecular complexes produced by activated neutrophile. NETs fight infection by immobilizing and killing bacteria, viruses and fungi, ultimately activating the innate immune response and coagulation. Abnormal NETs formation is associated with chronic autoimmunity and sterile and infectious inflammation (Boeltz et al., 2019).

- Herbal Medicine -

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care (Kamboj, 2000). This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998). According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994). The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) (Vickers and Zollman, 1999). Medical history from the beginning of time is filled with descriptions of persons who used herbs to heal the sick of the society. However, parallel to the onset of the industrial revolution we witnessed the rise of allopathic medicine. Herbal medicine was also an effective healing method, but was viewed less enthusiastically (Tirtha, 1998).

- Herbal products were discarded from conventional medical use in the mid-20th century, not necessarily because they were ineffective but because they were not as economically profitable as the newer synthetic drugs (Tyler, 1999). In the early 19th century, scientific methods become more advanced and preferred, and the practice of botanical healing was dismissed as quackery. In the 1960s, with concerns over the iatrogenic effects of conventional medicine and desire for more self-reliance, interest in “natural health” and the use of herbal products increased. Recognition of the rising use of herbal medicines and other non-traditional remedies led to the establishment of the office of Alternative Medicine by the National Institute of Health USA, in 1992. Worldwide, herbal medicine received a boost when the WHO encouraged developing countries to use traditional plant medicine to fulfill needs unmet by modern systems (Winslow and Kroll, 1998). The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Traditional medicine is the synthesis of therapeutic

experience of generations of practicing physicians of indigenous system of medicine. Traditional preparations comprise medicinal plants, minerals and organic matter etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atharvaveda, Charak Samhita and Sushruta Samhita. The herbal medicines / traditional medicaments have therefore been derived from rich traditions of ancient civilizations and scientific heritage (Kamboj, 2000).

III. PLANT PROFILE

- Balanites Ingudi –

Many underutilized tree species are good sources of food, fodder and possible therapeutic agents. Balanites ingudi (L.) Delile belongs to the Zygophyllaceae family and is popularly known as “desert date”, reflecting its edible fruits. Various parts of the plant are used in Ayurvedic and other folk medicines for the treatment of different ailments such assyphilis, jaundice, liver and spleen problems, epilepsy, yellow fever and the plant also has insecticidal, antihelminthic, antifeedant, molluscicidal and contraceptive activities (Yadav et al., 2010).

- Morphological description -

It is multibranched, spiny shrub or tree up to l0 m tall. Crown spherical, in one or several distinct masses. Trunk short and often branching from near the base. Bark dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8 cm long. Leaves with two separate leaflets; leaflets obovate, asymmetric, 2.5 to 6 cm long, bright green, leathery, with fine hairs when young. Flowers in fascicles in the leaf axils, and are fragrant, yellowish-green.

- Fruit and seed description –

Fruit isa rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. Pulp is bitter-sweet and edible. Seed is the pyrene (stone), 1.5 to 3 cm long, light brown, fibrous, and extremely hard. It makes up 50 to 60% of the fruit. There are 500 to 1 500 dry, clean seeds per kg.

- Flowering and fruiting habit –

Flowers are small, inconspicuous, hermaphroditic, and pollinated by insects. Seeds are dispersed by ingestion by birds and animals. The tree begins to flower and fruit at 5 to 7 years of age and maximum seed production is when the trees are 15 to 25 years old (Chothani and Vaghasiya, 2011)

IV. RESULT AND DISCUSSION

- Determination of percentage yield

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used.

Table 1 - % yield of Litsea glutinosa

Sr.no	Extracts	Colour/ texture	% yield (w/w)
1.	Pet. Ether	Dark black semisolid	1.2 %
2.	Hydroalcoholic	Brown solid	8.5 %

% Yield of pet. Ether and hydroalcoholic extract of Balanites aegyptiaca were found to be 1.2% and 8.5% respectively. The lower percentage yield of 1.2% for the pet ether extract suggests that this solvent may not be as effective in extracting compounds from Balanites aegyptiaca compared to the hydroalcoholic solvent. The relatively low yield could indicate that Balanites aegyptiaca contains a higher proportion of polar compounds, which are better extracted using a hydroalcoholic solvent. Conversely, the hydroalcoholic extract yielded 8.5%, indicating a more efficient extraction process with this solvent.

- Phytochemical screening of extract

Phytochemical screening of Balanites aegyptiaca extract could further elucidate the nature of its bioactive constituents. The presence of compounds can be indicative of potential medicinal properties. For instance, alkaloids often contribute to

pharmacological activities such as analgesic or anti-inflammatory effects, while flavonoids are renowned for their antioxidant and anti-inflammatory properties. The choice of solvents in the extraction process is crucial for capturing the full spectrum of phytochemicals present in the plant. Hydroalcoholic solvents, by virtue of their ability to dissolve both polar and non-polar compounds, can provide a more comprehensive phytochemical profile. The findings lay the foundation for more in-depth studies, aiming to isolate and identify specific phytochemicals, assess their biological activities, and explore the potential therapeutic applications of Balanites aegyptiaca in the realm of traditional medicine or as a source of novel pharmaceutical agents. Small amount of each extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml. The outcomes of the results are Discussed in the table 2

Table 2 – Phytochemical screening of extract of Balanites aegyptiaca

Sr. No	Constituent	Hydroalcoholic extracts	Observation
1.	Alkaloids Dragendroff's tests Hager's test	 +ve -ve	 Red precipitated Yellow but no precipitated
2.	Glycosides Legal's test	 -ve	 Dark brown colored
3.	Flavonoid Lead acetate Alkaline test	 +ve +ve	 Yellow precipitate Colorless
4.	Phenol Ferric chloride test	 +ve	 Black colored
5.	Proteins Xanthoproteic test	 -ve	 Brown colored
6.	Carbohydrate Fehling's test	 +ve	 Red colored Precipitate
7.	Saponin Foam test	 +ve	 Layer of Foam
8.	Diterpene Copper acetate test	 +ve	 Emerald green color
9.	Tannin Gelatin test	 -ve	 Brown colored

The phytochemical screening of Balanites aegyptiaca hydroalcoholic extract, as presented in Table 2, provides valuable insights into the diverse bioactive constituents inherent in the plant. The alkaloidal content, identified through Dragendroff's tests, exhibited positive results with the formation of red precipitates, respectively. This suggests the presence of alkaloids in the extract. Conversely, the absence of glycosides, indicated by the negative result in Legal's test, eliminates the possibility of dark brown coloration associated with glycoside compounds. Flavonoids were detected through Lead acetate and Alkaline tests, presenting a yellow precipitate and remaining colorless during observation. The positive reaction to the Ferric chloride test indicates the presence of phenolic compounds, manifested by a black coloration. Proteins, however, as determined by the Xanthoproteic test, were found to be absent, as evidenced by the lack of brown coloration.

Carbohydrates were positively identified through Fehling's test, leading to a red-colored precipitate. Saponins, detected through the foam test, resulted in a layer of foam formation. Diterpenes, revealed by the Copper acetate test, exhibited an emerald green coloration. Tannins, identified by the Gelatin Test, were found to be absent, as indicated by the lack of brown coloration. The comprehensive phytochemical profile obtained from this screening provides a foundation for further investigations into the medicinal and therapeutic potential of Balanites aegyptiaca, guided by the specific classes of phytoconstituents identified. These findings contribute to the broader understanding of the plant's chemical composition and support its potential

applications in traditional medicine or pharmaceutical research.

Results of estimation of total flavonoids and phenol content

- Total flavonoids content estimation (TFC)

The total flavonoid content, measured at a specific concentration per 100 milligrams of dried extract, signifies the abundance of flavonoids in Balanites aegyptiaca. Flavonoids are known for their antioxidant, anti-inflammatory, and antimicrobial properties, and their presence in the extract suggests that Balanites aegyptiaca may possess compounds that could contribute to these health benefits. The exact concentration provides a basis for Further comparison with established standards and for assessing the potential efficacy of the Extract in various applications. Total flavonoids content was calculated as quercetin Equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.031x – 0.014, R^2-0.998, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 3: Presents the preparation of a calibration curve for quercetin

Sr. No	Concentration	Absorbance
1.	5	0.134±0.003
2.	10	0.287±0.002
3.	15	0.466±0.005
4.	20	0.622±0.002
5.	25	0.779±0.001

antioxidant and anti-inflammatory properties. The data consists of various concentrations of quercetin (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 25 µg/ml) along with their corresponding absorbance values, measured as Mean+S.D (Standard Deviation). The observed increase in absorbance with increasing concentrations of quercetin suggests a linear relationship between the two variables. The information derived from the calibration curve is instrumental in determining the concentration of quercetin in unknown samples based on their absorbance values.

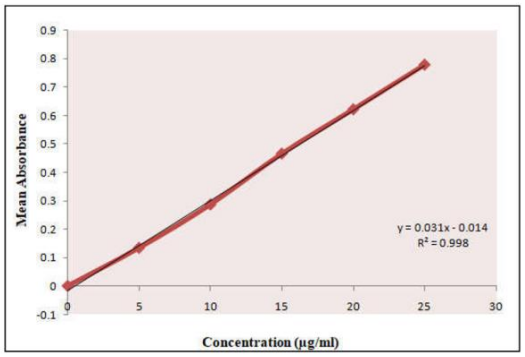

Fig 1 – Calibration curve of Quercetin

- Total phenol content estimation (TPC)

Similarly, the estimation of total phenol content is crucial for understanding the antioxidant potential of Balanites aegyptiaca. Phenolic compounds, with their antioxidant activity, play a significant role in protecting cells from oxidative stress. The measured content, expressed in milligrams per 100 milligrams of dried extract, reflects the concentration of these potentially bioactive compounds in the plant. Total phenol content was calculated as gallic acid equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.013x+ 0.003, R=0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

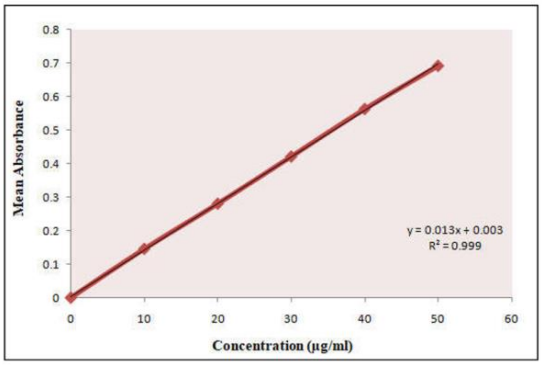

Fig 2– Graph of calibration curve of gallic acid

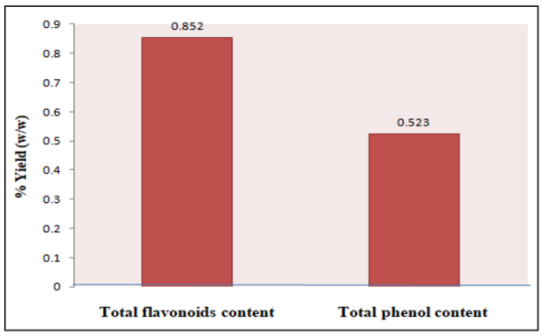

Fig 3 – Graph of total flavonoids and phenol content

The estimation of total flavonoids and phenol content in the hydroalcoholic extract of Balanites aegyptiaca, as depicted in Table 6.5, reveals valuable quantitative information about the presence of bioactive compounds in the plant. The total flavonoids content is determined to be 0.852 mg per 100 mg of dried extract. Flavonoids, with their well- documented antioxidant properties, play a significant role in various physiological functions and are often associated with potential health benefits. The measured flavonoids content indicates the extract's potential as a source of these bioactive compounds. Furthermore, the total phenol content in the hydroalcoholic extract is reported as 0.523 mg per 100 mg of dried extract. Phenolic compounds, known for their antioxidant and anti-inflammatory properties, contribute to the overall medicinal potential of plant extracts. The observed phenol content underscores the extract's richness in these compounds, further supporting its potential therapeutic applications. It is important to note that the quantification of these constituents provides a quantitative basis for understanding the potential health-promoting effects of Balanites aegyptiaca. However, to comprehensively evaluate its medicinal and therapeutic potential, further studies, such as bioassays and pharmacological investigations, are warranted. The presented data serve as a valuable starting point for researchers and contribute to the broader understanding of Balanites aegyptiaca's phytochemical Composition and potential applications in the field of natural products and medicine.

V. IN VIVO PHARMACOLOGICAL ACTIVITY

Methodology and In vivo anti-inflammatory activity of fruits extract of Balanites aegyptiaca

Animals

Wistar rats (150-200 g) were group housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25+2°C, 55-65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise- free room between 08.00 to 15.00 h. A separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India (Meshram et al., 2016).

Experimental designs.

Group-I: Carrageenan (1% w/v)

Group-II: Carrageenan fruits extract of Balanites aegyptiaca-100 mg/kg

Group-III: Carrageenan + fruits extract of Balanites aegyptiaca-200 mg/kg

Group-IV: Carrageenan + Indomethacin (10 mg/kg bw)

Carrageenan induced hind paw oedema

The anti-inflammatory activity was measured using carrageenan-induced rat paw oedema assay. The rats were divided into four groups of 6 animals each (plant extract was dissolved and administered per oral at different dose levels).

Group 1: was treated as control (0.1 ml of 1% (w/v) of was treated with carrageenan (1%w/v) in saline in the sub planter region of the right hind paw),

Group II: Carrageenan+ fruits extract of Balanites aegyptiaca-100 mg/kg.

Group III: Carrageenan fruits extract of Balanites aegyptiaca-100 mg/kg.

Group IV: Carrageenan + Indomethacin (10 mg/kg bw). Oedema was induced by injecting 0.1ml. of a 1% solution of carrageenan in saline into the sub plantar region of the right hind paw of the rats. The volumes of oedema of the injected and the contralateral paws were measured after the induction of inflammation using a plethysmograph (Arslan et al., 2011; Ou et al., 2019).

- Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean standard error of the mean (SEM). Data were analyzed by one-way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Table 4: Effect of fruits extract of Balanites aegyptiaca on paw edema induced by carrageenan in rats by different timelines

Groups	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr
Group I	0.1ml of 1%	3.7 ± 0.08	4.5 ± 0.08	4.5 ± 0.03	4.6 ± 0.10	5.4 ± 0.10
Group II	100 mg/kg	2.4 ± 0.07	2.5 ± 0.05	2.5 ± 0.12	2.8 ± 0.20	2.8 ± 0.10
Group III	200 mg/kg	1.8 ± 0.10	1.7 ± 0.05	1.6 ± 0.1	1.4 ± 0.25	1.2 ± 0.05
Group IV	10 mg/kg	1.2 ± 0.08	1.4 ± 0.05	1.3 ± 0.05	1.2 ± 0.04	1.05 ±0.06

VI. RESULTS

The paw edema measurements were taken at different time points after administering the treatments, such as 0 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours, and the values were recorded using techniques such as calipers or plethysmometry.

CONCLUSION

In conclusion, the study on Balanites aegyptiaca encompassed extraction, phytochemical screening, and evaluation of its anti-inflammatory activity. The hydroalcoholic extract demonstrated a substantial yield and was found to contain various bioactive compounds, including alkaloids, flavonoids, phenols, saponins, diterpenes, and carbohydrates. The quantification of total flavonoids and phenols provided additional evidence of the extract's richness in these pharmacologically relevant constituents.

The investigation into anti-inflammatory activity using the carrageenan-induced paw edema model revealed promising results. The Balanites aegyptiaca extract, particularly at higher doses, exhibited a significant reduction in paw edema, suggesting a potential anti- inflammatory effect. These observations align with traditional uses of Balanites aegyptiaca in folk medicine for managing inflammatory conditions.

Overall, the study provides a scientific basis for the traditional use of Balanites aegyptiaca as a medicinal plant with anti-inflammatory properties. The identified phytoconstituents and the demonstrated activity in the preclinical model lay the groundwork for future research.

ACKNOWLEDGEMENT

Authors are grateful to Principal and guide of Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal (M.P.), India, for providing encouragement and support for critically preparing this manuscript.

REFERENCES

[1] Kotas, M. E. & Medzhitov, R. Homeostasis, inflammation, and disease susceptibility. Cell 160, 816–827 (2015).

[2] Rock, K. L., Latz, E., Ontiveros, F. & Kono, H. The sterile inflammatory response. Annu. Rev. Immunol. 28, 321–342 (2009).

[3] Schett, G. & Neurath, M. F. Resolution of chronic inflammatory disease: universal and tissue-specific concepts. Nat. Commun. 9, 3261 (2018).

[4] Furman, D. et al. Chronic inflammation in the etiology of disease across the life span. Nat. Med. 25, 1822–1832 (2019).

[5] Zindel, J. & Kubes, P. DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. Annu. Rev. Pathol. Mech. Dis. 15, 493–518 (2020).

[6] Luster, A. D., Alon, R. & von Andrian, U. H. Immune cell migration in inflammation: present and future therapeutic targets. Nat. Immunol. 6, 1182– 1190 (2005).

[7] Rajendran, P. et al. The multifaceted link between inflammation and human diseases. J. Cell. Physiol. 233, 6458–6471 (2018).

[8] Darnell, M. & Mooney, D. J. Leveraging advances in biology to design biomaterials. Nat. Mater. 16, 1178–1185 (2017).

[9] Tang, D., Kang, R., Coyne, C. B., Zeh, H. J. & Lotze, M. T. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. Immunol. Rev. 249, 158–175 (2012).
[10] Cen, X., Liu, S. & Cheng, K. The role of toll-like receptor in inflammation and tumor immunity. Front. Pharmacol. 9, 878 (2018).
[11] El-Kenawi, A. & Ruffell, B. Inflammation, ROS, and mutagenesis. Cancer Cell 32, 727–729 (2017).
[12] Barber, G. N. STING: infection, inflammation and cancer. Nat. Rev. Immunol. 15, 760–770 (2015).
[13] Kurts, C. & Meyer-Schwesinger, C. Protecting the kidney against autoimmunity and inflammation. Nat. Rev. Nephrol. 15, 66–68 (2019).
[14] Neurath, M. F. Targeting immune cell circuits and trafficking in inflammatory bowel disease. Nat. Immunol. 20, 970–979 (2019).
[15] McDonald, B. & Kubes, P. Innate immune cell trafficking and function during sterile inflammation of the liver. Gastroenterology 151, 1087–1095 (2016). References Page 30
[16] Gargalionis, A. N., Basdra, E. K. & Papavassiliou, A. G. Mechanosignalling in tumour progression. J. Cell. Mol. Med. 22, 704 (2018).
[17] Knapik, D. M. et al. Mechanosignaling in bone health, trauma and inflammation. Antioxid. Redox Signal. 20, 970–985 (2014).
[18] Kreuger, J. & Phillipson, M. Targeting vascular and leukocyte communication in angiogenesis, inflammation and fibrosis. Nat. Rev. Drug Discov. 15, 125– 142 (2016).
[19] Kolaczkowska, E. & Kubes, P. Neutrophil recruitment and function in health and inflammation. Nat. Rev. Immunol. 13, 159–175 (2013).
[20] Németh, T., Sperandio, M. & Mócsai, A. Neutrophils as emerging therapeutic targets. Nat. Rev. Drug Discov. 19, 253–257 (2020).
[21] Finsterbusch, M., Voisin, M.-B., Beyrau, M., Williams, T. J. & Nourshargh, S. Neutrophils recruited by chemoattractants in vivo induce microvascular plasma protein leakage through secretion of TNF. J. Exp. Med. 211, 1307– 1314 (2014).
[22] Ng, L. G. et al. Visualizing the neutrophil response to sterile tissue injury in mouse dermis reveals a three-phase cascade of events. J. Invest. Dermatol. 131, 2058–2068 (2011).
[23] Baggiolini, M. Chemokines and leukocyte traffic. Nature 392, 565–568 (1998).
[24] Sallusto, F. & Baggiolini, M. Chemokines and leukocyte traffic. Nat. Immunol. 9, 949–952 (2008).
[25] Larouche, J., Sheoran, S., Maruyama, K. & Martino, M. M. Immune regulation of skin wound healing: mechanisms and novel therapeutic targets. Adv. Wound Care 7, 209–231 (2018).
[26] Atri, C., Guerfali, F. Z. & Laouini, D. Role of human macrophage polarization in inflammation during infectious diseases. Int. J. Mol. Sci. 19, 1801 (2018).
[27] Bäck, M., Yurdagul, A., Tabas, I., Öörni, K. & Kovanen, P. T. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. Nat. Rev. Cardiol. 16, 389–406 (2019). References Page 31
[28] Mantovani, A., Dinarello, C. A., Molgora, M. & Garlanda, C. Interleukin-1 and related cytokines in the regulation of inflammation and immunity. Immunity 50, 778–795 (2019).
[29] McInnes, I. B., Buckley, C. D. & Isaacs, J. D. Cytokines in rheumatoid arthritis — shaping the immunological landscape. Nat. Rev. Rheumatol. 12, 63–68 (2016).
[30] Yang, B., Chen, Y. & Shi, J. Reactive oxygen species (ROS)-based nanomedicine. Chem. Rev. 119, 4881–4985 (2019).
[31] Tejero, J., Shiva, S. & Gladwin, M. T. Sources of vascular nitric oxide and reactive oxygen species and their regulation. Physiol. Rev. 99, 311–379 (2019).
[32] Duffin, R. et al. Prostaglandin E2 constrains systemic inflammation through an innate lymphoid cell–IL-22 axis. Science 351, 1333–1338 (2016).
[33] Ramachandran, R., Altier, C., Oikonomopoulou, K. & Hollenberg, M. D. Proteinases, their extracellular targets, and inflammatory signaling. Pharmacol. Rev. 68, 1110–1142 (2016).
[34] Daniel, C. et al. Extracellular DNA traps in inflammation, injury and healing. Nat. Rev. Nephrol. 15, 559–579 (2019).

[35] Boeltz, S. et al. To NET or not to NET: current opinions and state of the science regarding the formation of neutrophil extracellular traps. Cell Death Differ. 26, 395–408 (2019).
[36] Alschuler L, Benjamin SA, Duke JA (1997). Herbal medicine - what works, what is safe. Patient Care, 31, 48-103.
[37] Bensoussan A, Talley NJ, Hing M, et al (1998). Treatment of irritable bowel syndrome with Chinese herbal medicine: a randomized controlled trial. JAMA, 280, 1585-9.
[38] Bhatt AD and Bhatt NS (1996). Indigenous drugs and liver disease. Indian J Gastroenterol, 15, 63-7.
[39] Boullata JI and Nace AM (2000). Safety issues with herbal medicine. Pharmacotherapy, 20, 257-69.
[40] Brevoort P (1998). The booming US botanical market. A new overview. Herbal Gram, 44, 33-44.
[41] Carter AJ (1999). Dwale: an anesthetic from old England. BMJ, 319, 1623-6. References Page 32
[42] Chattopadhyay MK (1996). Herbal medicines. Current Science, 71, 5.
[43] Chattopadhyay MK (1997). Herbal medicine - some more reports. Current Science, 72, 6.
[44] Cox PA (2000). Will tribal knowledge survive the millennium? Science, 287, 44-5.
[45] Cragg GM, Newmann DJ, Snader KM (1997). Natural product in drug discovery and development. J Nat Prod, 60, 52-60.
[46] De Smet PAGM (1995). Should herbal medicines-like product be licensed as medicines? BMJ, 310, 1023-4.
[47] DeSmet PAGM (1997) Adverse effect of herbal remedies. Adverse Drug Reactions Bulletin, 183, 695-8.
[48] Dhuri KD, Vaidya VA, Vaidya AD, et al (2000). Stress and Ayurveda: Selye Mehata Dialogue in context of the current findings. JAPI, 48, 428-31.
[49] Dwyer J and Rattray D (1993). Anonymous. Plant, People and Medicine. In Magic and Medicine of Plant. Reader's Digest general book, pp 48-73.
[50] Ernst E (2000). Herbal medicines: where is evidence? BMJ, 321, 395-6.
[51] Evans M (1994). A guide to herbal remedies. Orient Paperbacks.
[52] Gesler WM (1992). Therapeutic landscape: medicinal issue in light of the new cultural geography. Soc Sci Med, 34, 735-46.
[53] Gottlieb S (2000). Chinese herb may cause cancer. BMJ, 320, 1623A.
[54] Gottlieb S (2000). US relaxes its guidelines on herbal supplements. BMJ, 320, 207.
[55] Graham-Brown RA, Bourke JF, Bumphrey G (1994). Chinese herbal remedies may contain steroids. BMJ, 308, 473.
[56] Greensfelder L (2000). Alternative medicine. Herbal product linked to cancer. Science, 280, 1946.
[57] Gupta LM and Raina R (1998). Side effects of some medicinal plants. Current Science, 75, 897-900.
[58] Halt M (1998). Mould and mycotoxins in herbal tea and medicinal plant. Eur J Epidemiol, 14, 269-74.
[59] Harries AD and Cullinan T (1994). Herbis et orbis: the dangers of traditional eye medicine. The Lancet, 344, 1588. References Page 33
[60] Harvey AL (1999). Medicines from nature: are natural product still relevant to drug discovery? Trends Pharmacol Sci, 20, 196-8.
[61] Holland BK (1994). Prospecting for drugs in ancient text. Nature, 369, 702.
[62] Jain SK and DeFilipps RA (1991). Medicinal plants of India. Reference Publication, Inc. Jayaraman KS (1996). "Indian ginseng" brings royalties for tribe. Nature, 381, 182.
[63] Kamboj VP (2000). Herbal Medicine. Current Science, 78, 35-9