Formulation and Evaluation of Herbal Buccal Patch for Dental Diseases

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Abstract: The present study deals with an approach in formulation and evaluation of buccal patch with the incorporation of herbal extracts. The present study deal with buccal patches, were prepared with herbal (*neem and kanuga*) extracts and polymers such as hydroxyl polymer methyl cellulose (HPMC) in respect to solvent such as ethanol and propylene glycol as plactizier. Buccal patches were formulated using solvent casting method with herbal extracts and prepared patches were characterized in terms of film thickness, folding endurance, surface pH, swelling index, moisture content and *In-vitro* diffusion studies. *In-vitro* anti-inflammatory studies were performed by using "human red blood cell membrane stabilization" method and *In-vitro* anti-bacterial studies were also performed by using "cup and plate method" with the use of herbal extracts. The combination of *azadirachta indica* and *pongamia pinnate* possess the highest anti-inflammatory and anti-bacterial activity when compared with individual extracts.

Key Words: Buccal patchs, anti-inflammatory, anti-bacterial, diffusion and herbal extracts.

INTRODUCTION

Dental cavities and gums diseases are very common. According to World Health Organisation between 60-90% of school children have at least one dental cavity. Nearly 100% of adults have at least one dental cavity. Between 15 and 20% of adults ages 35 to 44 have severe gum disease. About 30% of people around the world ages 65 to 74 don't have any natural teeth left.[1]
Selection of drug for BDDS: Molecular weight should be less than 1000Da. It should be having both nature *i.e.,* hydrolipophilic type, non-irritant to mucosa and Drugs that degrades in GIT.

MATERIALS AND METHODS

Materials: *Azadirachta indica* (Neem), *Pongamia pinnata* (Kanuga), Ethanol, Methyl cellulose, Propylene glycol, Sorbitol, Blood, Hyposaline, Alsevers solution, Agar gel, Bacteria, Diclofenac and Paracetamol.

Method: The selected plant parts of *azadirachta indica (*neem), *pinnate pongamia* (kanuga) were taken from the grounds of Malla Reddy Pharmacy College. The stems of both neem and kanuga are cleaned with fresh water and are air dried for few days and then they are powdered into fine mixture. The obtained powders are stored in air tight containers at room temperature and preserved.

Extraction: Fresh neem and kanuga stems were collected and they were washed thoroughly with normal water to remove dust particles from the surface of stems. Then they are again washed with distilled water and they were shed dried. After drying, leaves were grinded until coarse powder is obtained. Then significant amount of the powdered leaves (200gm) were weighed and they were macerated in 1000ml of ethanol for 7 days. Solvent containing the extract was decanted and was concentrated using a rotary evaporator to get the crude extract. The concentrated extract was then dried under room temperature and it was used further for preparing muco-adhesive patches.[2]

Preparation of buccal patches: The technique employed for preparing muco-adhesive buccal patches was solvent casting technique. They were prepared by dissolving suitable concentration of polymer such as 600mg in 5ml of ethanol and calculated amount of the extract (40mg) was dissolved in another 5ml of ethanol and this mixture was added to the polymer mixture followed by the addition of 0.5ml of sweetening agent. Then 0.5ml of the plasticizer was added to all formulations and was further sonicated to remove all entrapped air bubbles. Then, they were transferred to a petridish and allowed to dry under room temperature by placing a funnel in an inverted position over the petridish for 24 hours. After that, all the patches were studied for further characterizations.[3]

Pre-formulation studies:

Solubility studies: Accurately weighed 10mg of extracts (neem, kanuga, combination of two extracts) were taken and the extracts were dissolved in different solvents such as ethanol, water, 6.8phosphate buffer and 7.4phosphate buffer. The mixtures were shaken for 24hrs at regular intervals. The solutions were filtered by using whattman's filter paper. The filtered solutions were analysed at suitable nm.[4], [5]

Determination of Lamda (λ_{max}) max:

Neem: A solution of neem containing the concentration of 100µg/ml was prepared in 6.8 phosphate buffer and UV spectrum was taken. The solution was scanned in the range of 400-650nm.

Kanuga: A solution of kanuga containing the concentration of 100µg/ml was prepared in 6.8 phosphate buffer and UV spectrum was taken. The solution was scanned in the range of 400-700.

Combination (Neem + Kanuga): A solution of neem and kanuga containing the concentration of 100µg/ml was prepared in 6.8 phosphate buffer and UV spectrum was taken. The solution was scanned in the range of 400-700.

Construction of standard graphs:

Neem: 100mg of neem extract was taken accurately in 100ml volumetric flask. It was dissolved in 10 ml of ethanol & volume was made up to 100ml with 6.8 phosphate buffer to give 1000µg/ml which is stock-1. From stock-1,10ml was transferred into 100ml volumetric flask volume was made up to 100ml with 6.8 phosphate buffer which gives stock-2. From the stock-2 serial dilutions were made to get concentration 2, 4, 6, 8, 10µg/ml of neem respectively. The absorbance was measured against 6.8 phosphate buffer as blank at 510 nm using UV visible spectrophotometer at 510nm.[6]

Kanuga: 100mg of kanuga extract was taken accurately in 100ml volumetric flask. It was dissolved in 10 ml of ethanol & volume was made up to 100ml with 6.8 phosphate buffer to give 1000µg/ml which is stock-1. From stock-1,10ml was transferred into 100ml volumetric flask volume was made up to 100ml with 6.8 phosphate buffer which gives stock-2. From the stock-2 serial dilutions were made to get concentration 2, 4, 6, 8, 10µg/ml of kanuga respectively. The absorbance was measured against 6.8 phosphate buffer as blank at 500nm using UV visible spectrophotometer at 500nm.[7]

Combination (Neem and Kanuga): 100mg of combination extract was taken accurately in 100ml volumetric flask. It was dissolved in 10 ml of ethanol & volume was made up to 100ml with 6.8 phosphate buffer to give 1000µg/ml which is stock-1. From stock-1 transferred 10ml into 100ml volumetric flask volume was made up to 100ml with 6.8 phosphate buffer which gives stock-2. From the stock-2 serial dilutions were made to get concentration 2, 4, 6, 8, 10µg/ml of combination respectively. The absorbance was measured against 6.8 phosphate buffer as blank at 560 nm using UV visible spectrophotometer at 560nm

Evaluation test for buccal patches:[8]

Thickness measurement: Film thickness was measured using Vernier Callipers from all sides at different position and the average value was noted.

Surface pH: Surface pH of patches was determined by placing pH paper on the surface of patches.

Folding Endurance: Folding endurance of buccal patches was determined by folding each patch at the same place repeatedly until it breaks. Number of times the patches can folded until it breaks gives the value of folding endurance and the average value was noted.

Swelling index: Patches of 1cm² each were allowed to swell in 2% agar solution in a clean, dry petridish for two hours consecutively. Each patch of size was cut and their initial weight was noted. Then they were allowed to swell for 5min in 20ml of distilled water. Patches were than taken out, dried and weighed.

Percentage of swelling was noted using the following formula:

Swelling index (SI) =final weight-initial weight/initial weight*100

Moisture content: all the patches of size1cm*1cm was initially weighed. They were placed in desiccators containing calcium chloride and the internal humidity was maintained. After 72 hours, all patches were collected back and reweighed. Average value was noted using the following formula.
Percentage moisture content (PML) = Initial weight-final weight/initial weight*100

In-vitro diffusion studies**:** The *in-vitro* drug diffusion study was carried out using open ended cylinder method with the use of a membrane that is semi permeable. The dialysis membrane was activated by immersing it into the water. The membrane of appropriate size was tied to one end of the open ended cylinder which acted as the donor compartment.
Then the cylinder was dipped into the diffusion medium which acted as the receptor compartment. Phosphate buffer of pH 6.8 was used as the diffusion medium. Patches of appropriate sizes were then placed in the donor compartment and they were kept separated from the receptor compartment using the dialysis membrane. Temperature was maintained at 37˚C at 50 rpm. 10 ml of the sample was withdrawn after every half an hour for 3 consecutive hours and simultaneously the receptor compartment that is the diffusion medium was replaced with the fresh buffer. The absorbance was determined using UV spectrophotometer at 510nm.[9]

Anti-inflammatory activity:[10], [11]
Human red blood cell membrane stabilization method: Blood was collected from healthy volunteers. Collected blood was mixed with equal volume of 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.4% Nacl in water (Alavert solution) Blood was centrifuged at 3000rpm and packed cells were washed with isosaline 0.85%, pH-7.2 and 10% volume suspension was made with isosaline. Assay the mixture contained. 1ml of phosphate buffer (0.15ml, Ph-7.4); 2ml of hyposaline, 0.5ml of HJRBC suspension and diclofenac as reference drug. Instead of hyposaline 2ml od distilled water was used as suspension for control. All the assay mixture were collected at 37˚C for 30 min and centrifuged. % hemoglobin content in the supernatant solution is estimated by spectrophotometer at 560nm. %hemoglobin was calculated by assuming the hemolysis produced in the presence of distilled water as 100%.
Percentage of HRBC membrane stabilization or protection was calculated using the following formula:
%Inhibition of hemolysis = {(optical density of control - optical density of test samples)/ optical density of control} x100

Anti-bacterial activity - Cup and plate method:[12]
Preparation of the medium: The medium were prepared by dissolving the specific quantity of the agar in purified water by heating on a water bath and were dispensed in 100ml volumetric conical flask. The conical flasks were closed with cotton plugs and were sterilized by auto claving at 121º C for 15min. After sterilization, the volumetric flask was kept in an inclined position for solidification. The test organisms were transferred to the agar medium from the supplied cultures with the help of an inoculating loop in aseptic conditions. The loop was burned after each transfer of microorganism to avoid contamination very carefully. Then, the contents of the conical flasks were pored aseptically into sterile petridish is allowed to solidify. These sterilized medias were used to subculture the bacterial culture.

Procedure**:** Each Petridish was filled to a depth of 4-5 mm with a nutrient agar medium that was previously inoculated with the suitable test organism, and then allowed to solidify. The petridish were specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium in uniform thickness. To each portion one cylindrical cavity was made in medium with the help of sterile borer. One for test organism and the other for standard. The petridishes were incubated at 37ºc for 24hrs. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained by the test sample was compared with that produced by standard penicillin

RESULT

Anti-inflammatory activity: The stabilization of liposomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constitutes of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation and produce several disorders. The HRBC membrane stabilization has been used as a method to study the in-vitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane. The non-steroidal anti-inflammatory drugs (NSAIDS) act either by inhibiting these enzymes or by stabilizing the lysosomal membrane.

Anti-bacterial activity: Plant extracts have been used for many thousands of years. It is necessary to investigate those plants which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial diseases. *In vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. In our study, neem, kanuga and combination drug exhibited strong activity against the selected bacterial strains. Several studies have shown that neem and kanuga had strong and consistent inhibitory effects against various bacterial strains. The antibacterial activity has been attributed to the presence of some active constituents in the plant extracts. Both Gram-positive bacteria and gram-negative bacteria showed maximum resistant in combination drug when compared to the individual extracts.

Table 1: Composition of herbal buccal patches of kanuga and neem extracts.

S.No	Ingredients	F1	F2	F3
1	Neem extract	40mg	-	20mg
2	Kanuga extract	-	40mg	20mg
3	HPMC	600mg	600mg	600mg
4	Ethanol	10ml	10ml	10ml
5	Propylene glycol	0.5ml	0.5ml	0.5ml
6	Sorbitol	0.5ml	0.5ml	0.5ml

Table 2: Phytochemical test of kanuga and neem extracts.

Test	Neem	Kanuga
Alkaloids	+ve	+ve
Carbohydrates	+ve	+ve
Flavonoids	+ve	+ve
Glycosides	+ve	+ve
Steroids	-ve	+ve
Terpenoids	+ve	+ve
Fats	-ve	+ve

Table 3: Evaluation test of herbal buccal patch of kanuga and neem

Formulations	Thickness	Folding endurance	Surface pH	Swelling index	Moisture content
F1	0.110mm	Flexible	7	25%	negligible
F2	0.110mm	Very flexible	7	29%	negligible
F3	0.112mm	Very flexible	8	28%	negligible

Table 4: Diffusion studies of herbal buccal patch of kanuga and neem

Time (min)	F1	F2	F3
0	0	0	0
30	49.12	53.22	65.39
60	57.32	62.55	69.33
90	70.61	70.12	73.45
120	77.42	78.31	81.51
150	82.11	86.36	86.31
180	87.38	91.45	93.26

An Anti-inflammatory activity was performed by using HRBC Membrane stabilizing method of neem, kanuga individually and also combined extracts. The combined extract showed highest percentage inhibition of haemolysis (69.13%) when compared to the individual extracts of neem (59.89% 63.19%), kanuga (63.19 %).

Table 5: Effect of the ethanolic extract of neem and kanuga on heat induced haemolysis of erythrocyte

Treatment	Concentration (µg/ml)	Absorbance at 560nm	% inhibition of haemolysis
Control	_	0.049±0.020	_
Diclofenac (Standard)	200	0.088±0.003	86.81%
	400	0.091±0.002	88.32%
Neem	200	0.077±0.004	57.16%
	400	0.078±0.010	59.89%
Kanuga	200	0.079±0.009	61.11%
	400	0.080±0.003	63.19%
Combination (Neem + Kanuga)	200	0.082±0.004	67.44%
	400	0.083±0.007	69.13%

Anti-bacterial activity was performed using cup and plate method of neem, kanuga, and combined extracts. The combined extracts showed highest zone of inhibition with *E.coli* 24±0.44mm, and *Bacillus Subtillis* 23±0.36mm. In comparison with individual extracts of neem *with E.coli* (16±0.30mm), *Bacillus Subtillis* (19±0.45mm) and kanuga with *E.coli* (20±0.42mm) *Bacillus Subtillis* (22±0.31mm).

Table 6: Effect of anti-bacterial activity of kanuga and neem

Plant	Penicillin (zone of inhibition)	*E.coli* (zone of inhibition)	*Bacillus Subtillis* (zone of inhibition)
Neem	26±0.52mm	16±0.30mm	19±0.45mm
Kanuga	26±0.52mm	20±0.42mm	22±0.31mm
Combination (Neem + Kanuaga)	26±0.52mm	24±0.44mm	23±0.36mm

Fig. 1: Dried extracts of kanuga and neem

Fig. 2: Formulation of buccal patch of neem

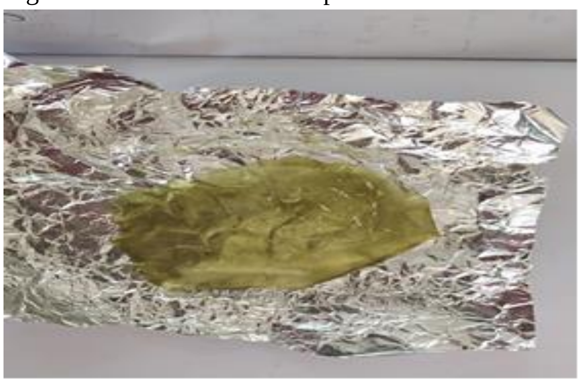


Fig. 3:- Formulation of buccal patch of kanuga

Fig. 4: Formulation of buccal patch of neem and kanuga combination

Fig. 5: HRBC membrane stabilization method of buccal batch of formulation F1 – F3

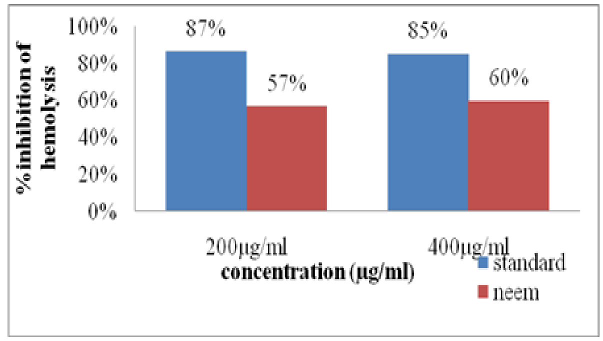


Fig. 6: Membrane stabilizing activity of neem extract

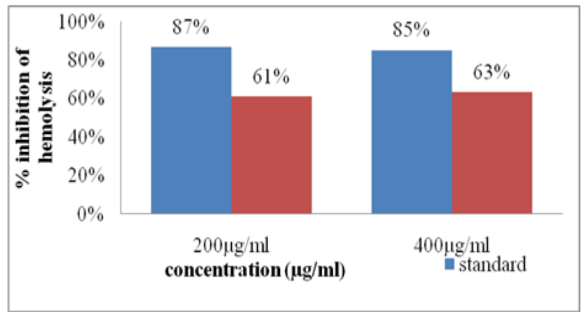


Fig. 7: Membrane stabilizing activity of kanuga extract

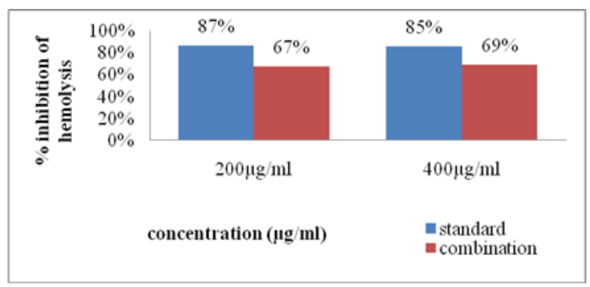
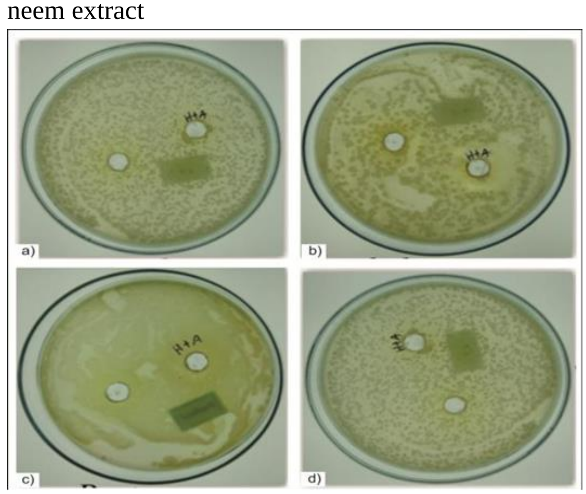


Fig. 8: Membrane stabilizing activity of kanuga and neem extract



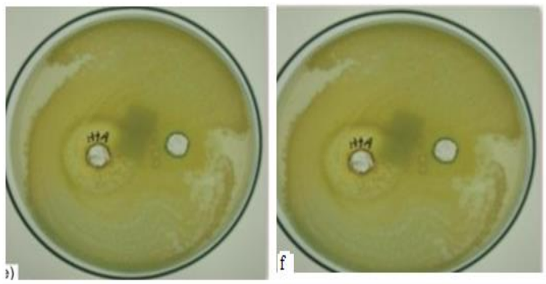


Fig. 9: Membrane stabilizing activity of kanuga and neem extract
a) Neem (E.Coli)
b) Kanuga (E.Coli)
c) Combination (E.Coli)
d) Neem (Bacillus)
e) Kanuga (Bacillus)
f) Combination (Bacillus)

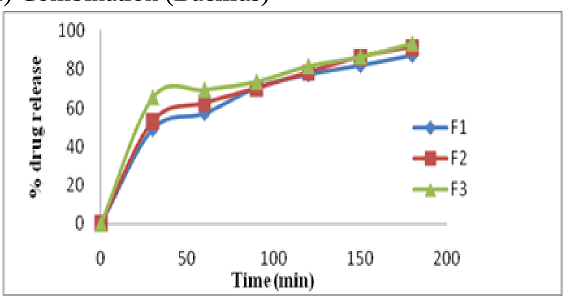


Fig.10: Diffusion studies of buccal batch of formulation F1 – F3

CONCLUSION

From the above studies, it could be concluded that the ethanolic extract of Azadirachta indica and Pongamia pinnate showed both the anti-inflammatory and anti-bacterial activity and showed its use in bacterial and inflammatory diseases. From the present study, it is concluded that combination of *Azadirachta indica* and *Pongamia pinnate* possess the highest anti-inflammatory and anti-bacterial activity when compared with individual extracts.

REFERENCE

[1] “Oral health.” https://www.who.int/news-room/fact-sheets/detail/oral-health (accessed Sep. 20, 2022).

[2] N. Hashim, S. Abdullah, L. S. Hassan, S. R. Ghazali, and R. Jalil, “A study of neem leaves: Identification of method and solvent in extraction,” *Mater. Today Proc.*, vol. 42, pp. 217–221, Jan. 2021, doi: 10.1016/J.MATPR.2020.11.726.

[3] A. Semalty, M. Semalty, and U. Nautiyal, “Formulation and Evaluation of Mucoadhesive Buccal Films of Enalapril Maleate,” *Indian J. Pharm. Sci.*, vol. 72, no. 5, p. 571, Sep. 2010, doi: 10.4103/0250-474X.78522.

[4] S. M. Habibur and R. M. Pharm, “‘INVESTIGATION OF ESSENTIAL OILS AS NATURAL PRESERVATION SYSTEM FOR DEVELOPMENT OF LIPID BASED NUTRICOSMETICS,’” 2017.

[5] “Preformulation Studies: Solubility analysis - Pharmapproach.com.” https://www.pharmapproach.com/preformulation-studies-solubility-analysis/ (accessed Nov. 01, 2022).

[6] D. F. Coelho *et al.*, “Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity, and the role of exercise on these parameters,” *Brazilian J. Med. Biol. Res.*, vol. 44, no. 10, pp. 966–972, 2011, doi: 10.1590/S0100-879X2011007500107.

[7] P. Parashar *et al.*, “Evaluating Anti-Inammatory Potential of Etodolac Co-Crystals: Nitric Oxide, IL-6 And TNF-a As Potential Markers,” 2021, doi: 10.21203/rs.3.rs-592061/v1.

[8] R. I. Prasanna and K. U. Sankari, “Design, evaluation and in vitro - in vivo correlation of glibenclamide buccoadhesive films,” *Int. J. Pharm. Investig.*, vol. 2, no. 1, p. 26, 2012, doi: 10.4103/2230-973X.96923.

[9] D. F. de Andrade, C. Zuglianello, A. R. Pohlmann, S. S. Guterres, and R. C. R. Beck, “Assessing the In Vitro Drug Release from Lipid-Core Nanocapsules: a New Strategy Combining Dialysis Sac and a Continuous-Flow System,” *AAPS PharmSciTech*, vol. 16, no. 6, p. 1409, Dec. 2015, doi: 10.1208/S12249-015-0330-0.

[10] S. B. Divya, L. N. Mahammed, and T. G. Rani, “In-Vitro Anti-Inflammatory Activity of Combined Extracts of Selected Plants,” *Int. J. Adv. Pharm. Biotechnol. J. homepage*, vol. 6, no. 1, pp. 1–03, 2020, doi: 10.38111/ijapb.20200601001.

[11] R. Paramaguru, K. Jagadeeshwar, C. B.

Mahendra Kumar, * N Armstrong, and V. Raj, “Evaluation of anti-inflammatory activity on the leaves of Filicium decipiens in experimental animal models,” *J. Chem. Pharm. Res*, vol. 3, no. 3, pp. 243–247, 2011.

[12] “Medical Microbiology Laboratory (culture media preparation).” https://www.slideshare.net/HusseinAltameemi2/medical-microbiology-laboratory-culture-media-preparation (accessed Nov. 01, 2022).