Formulation of Herbal Bath Soap from *Vitex negundo* Leaf Extract

Kandasamy Ruckmani[1,2*], Rengasamy Krishnamoorthy[1], Subramani Samuel[1], Henry Linda Jeeva Kumari[1,2]
[1]Department of Pharmaceutical Technology, Bharathidasan Institute of Technology,
Anna University, Tiruchirappalli 620 024, Tamil Nadu, India.
[2]National Facility for Drug Development for Academia, Pharmaceutical and Allied Industries, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli 620 024, Tamil Nadu, India.
***Corresponding Author: Email: hodpharma@gmail.com; Telephone: 0431-2407978**

ABSTRACT

Bacterial and fungal skin infections are most common amongst people, requiring significant attention for treatment, and also to maintain a healthy skin thereafter. The herbal plant extracts are known to possess various potentials like anti-inflammatory, antibacterial and antifungal properties which are explored for ages and incorporated into various forms, for human use. One such usage is an herbal soap formulation that is used not only for treating microbial infections, but also for using it on a daily basis. The aim and objective of the present study is to formulate an herbal bath soap using *Vitex negundo* Linn.(Verbenaceae) leaf extract which is of ethnic and dermatological importance and explore its antimicrobial properties. *Vitex negundo* leaves were extracted with methanol using Soxhlet apparatus and the methanolic leaf extract was examined for the functional groups of its active constituents by Fourier Transform Infra-Red (FT-IR) spectroscopy. The extract was then used for soap-making by the basic saponification reaction and the formulated herbal bath soap was further subjected to chemical characterizations such as estimation of saponification value, total fatty matter, moisture content and pH with the resulting values as 395.52 mg/mL, 70%, 6.23 % and 9.67 respectively. The formulation was found to be a stable solid without any colour change and categorized as Grade 2 soap. The antibacterial and antifungal activities of the formulated soap were performed which was found to be comparatively higher than that of the commercial antibacterial and antifungal soaps respectively as controls. The microbial strains used were of significant importance as the human skin is prone to such infections that arise from blisters, wounds and other eruptions. Hence, *Vitex negundo* extract is best suited for herbal soap preparation, for treating various skin infections and also for using it as regular bath soap.

KEYWORDS: *Vitex negundo*, saponification value, total fatty matter, moisture content, antibacterial, antifungal

1. INTRODUCTION

Plants with medicinal properties are being used as a traditional medicine from times immemorial. The extract from the leaves, stem and roots of various medicinal plants have been employed as a natural remedy in curing various ailments and diseases. Even though many plant based products have been replaced by synthetic chemicals, the safety and efficacy of ayurvedic products could not find their match. Besides having high nutritional value, many herbals are found to possess anti-bacterial (Perumal and Ignacimuthu, 2000), anti-oxidant, anti-microbial (Alade and Irobi, 1993), cytotoxicity of diseased conditions, anti-rheumatic (Ayo et al., 2007), anti-inflammatory (Dharmasiri et al., 2003), hypotensive (Riehemann et al., 1999), anti-diabetic, anti-hemorrhagic, anti-spasmodic (Shah et al., 2006), anti-helminthic (Guarrera, 1999) and anti-diuretic (Zaoui et al., 1999) properties. Natural products could be found in the treatment of almost all diseases and skin problems owing to their high medicinal value, cost-effectiveness, availability and compatibility (Solanki, 2011; Saikia et al., 2006). The active constituents responsible for such medicinal values are isolated and employed topically as creams, soaps, oils and ointments for treating skin related ailments like acne (Batubara et al., 2009), wounds (Biswas and Mukherjee, 2003; Marwah et al., 2007), eczemas, ring-worms, as an anti-microbial agent (Kareru et al., 2010) and for cosmetic purposes (Gray and Flatt, 1999). The plants like *Cassia alata* (Benjamin and Lamikanra, 1981), *Acalypha wilkesiana*, *Acacia senegal*, *Phyllanthus emblica* (Chaudhuri, 2002) are employed in skin care. The medicinal properties of the plants are being exploited in various formulations both in medical terms and cosmetic series.

Vitex negundo Linn., belonging to the family Verbenaceae, is an aromatic shrub distributed throughout India. The major phytochemical constituents present in the *Vitex negundo* leaf extract are Protocatechoic acid, Oleanolic acid, Flavonoids, Angusid, Casticin, Vitamin-C, Nishindine, Gluco-nonital, p-hydroxybenzoic acid and Sitosterol. It is found to have very good anti-oxidant (Tiwari and Tripathi, 2007), anti-fungal (Sathiamoorthy et al., 2007), anti-microbial, anti-inflammatory, analgesic (Telang et al., 1999), anti-histamine and mosquito repellent properties (Karunamoorthi et al., 2008). It has been also shown that *Vitex negundo* possess a neutralizing ability for the venoms of *Vipera russellii* and *Naja kaouthia*. The root extracts neutralized haemorrhage, coagulant, defibrinogenating and inflammatory activity caused by the snake venom (Alam and Gomes, 2003). The various available formulations of *Vitex negundo* plant extracts are: Sustained release tablet (Rheumatoid Arthritis), Leaf extract syrup (malarial fever), Capsules (anti- inflammatory activity), Notchi seed oil (anti- inflammatory activity), Ointment and Gel. The present study focuses on a novel soap formulation with the methanolic leaf extract of *Vitex negundo* having antibacterial and anti-fungal properties, which can also be used as regular bath soap.

2. MATERIALS AND METHODS

2.1. Materials: Coconut oil for soap-making was purchased from the local market. Culture media for the cultivation of bacteria were from Hi-media, Mumbai, India. Clinical pathogens were obtained from local hospitals. All other reagents used were of Analytical Grade.

2.2. Preparation of the Extract: The fresh leaves of *Vitex negundo* were obtained from Mandaiyur village near Anna University campus, Tiruchirappalli and authenticated in the Department of Botany, St. Joseph's College of Arts and Science, Tiruchirappalli and was deposited in their herbarium with voucher specimen number SS01. The leaves were thoroughly washed, air-dried and powdered. About 150 g of air dried coarse powdered leaves were soaked with petroleum ether for 2 days to remove the waxes present in the leaves. Then it was subjected to soxhlet extraction with methanol as solvent. Then the extract was concentrated by distillation and the solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of methanolic extract were noted. The extract was analysed for the major phytochemical constituents.

2.3. FTIR spectral studies: FTIR spectrophotometer was performed to assess the functional groups of the leaf extract. The hydraulic pellet press method was followed. Samples were taken in 1:100 ratios with KBr and mixed uniformly in a porcelain dish to prepare the pellets. The transmittance was recorded between 4000 and 400 cm^{-1} on FTIR spectrophotometer **(Jasco FT/IR-6300).** The functional groups present in the leaves were identified from the spectra.

2.4. Estimation of Saponification value: Saponification value gives us an idea on the amount of lye needed to make soap. The procedure to determine the saponification value is as follows: 1 g of oil was weighed and transferred into a round bottom flask. 20 ml of 0.5 N alcoholic caustic potash was then added to it. Without oil was also set for blank titration. Both were refluxed in round bottomed flasks for 1 h. After reflux, both the round bottomed flasks were allowed to cool. Both the samples were titrated using 0.5 N HCl with phenolphthalein indicator. Disappearance of pink colour was noted which indicates the end point. These values were noted to determine the saponification value using the formula, Saponification Value = (Titre value of blank in ml – Titre value of sample in ml) x Normality of KOH x equivalent wt of KOH)) /1g of oil.

2.5. Basic saponification reaction: The basic saponification reaction for the production of soap is the reaction between a neutral fatty acid and alkali to form soap and glycerol. Hence, coconut oil as a neutral fat and lye as an alkali has been used here for the basic saponification reaction as follows: 10.0 g of a fatty acid or oil (Coconut oil) was taken in a beaker. In another beaker, 12.5 ml of ethanol and 12.5 ml of deionized water were taken along with 3.5 g of alkali (Sodium hydroxide) pellets and stirred until completely dissolved. This solution was then added to the beaker in which oil was taken. After stirring for a while, this was kept on a hot plate, setting to medium/low heat, stirring for about 20-30 minutes until the smell of fat or oil disappears and the oil dissolves forming a homogenous solution. The mixture was allowed to cool and then the soap was filtered using Buchner funnel and Whatmann No.1 filter paper. 300 ml of a saturated sodium chloride solution was allowed to pass through the funnel for salt washing to remove any impurities. Then acid washing was done by passing 5 ml of 0.1N of dilute Hydrochloric acid twice. The soap was allowed to solidify for an hour to get the consistency. Two soaps were prepared in a similar manner, one for lower and one for higher concentrations of the formulation.

2.6. H**erbal Soap preparation:** The two solidified basic soaps were immediately broken down to smaller pieces and melted in a water bath. The weight of the methanolic *Vitex negundo* leaf extract was calculated based on the herbal content of commercial herbal bath soap. The extract was taken in lower and higher concentrations and added to the respective melted soaps along with 5 ml of ethanol. 0.033 g stearic acid was added in hot water, stirred and then added to the melted soap. 0.033g of TiO_2 was added to the melted glycerine soap and stirred. 2 ml of Lemongrass oil was added and stirred. Then, 1 g of Sodium Lauryl Sulphate was prepared in 5 ml distilled water and added to the mixture in drops. The respective soaps were gently mixed for about 30 minutes which were then moulded into separate circular moulds. The soaps were allowed to solidify at room temperature until set and kept under physical observation for any characteristic changes.

2.7. Chemical characterizations: The formulated dried herbal bath soap (lower concentration) was further evaluated and characterized for pH, Total Fatty Matter (TFM) and moisture content. pH of the soap was determined by touching the pH strip to the freshly prepared soap and also by dissolving 1 g in 10 ml water when using pH meter. TFM estimation was carried out by reacting soap with acid in the presence of hot water and measuring the fatty acids obtained. About 10 g of the formulated soap (lower concentration) was weighed and 150 ml distilled water was added and heated. The soap was dissolved in 20 ml of 15 % H_2SO_4 while heating until a clear solution was obtained. Fatty acids on surface of the resulting solution was solidified by adding 7 g bees wax and re-heated. The set up was allowed to cool to form cake. Cake was removed and blotted to dry and weighed to obtain the TFM using the formula, % TFM= (Weight of the oil – weight of the wax) in g / Weight of the soap in g * 100. The moisture content estimation was used to determine the percentage of water in the soap by drying the soap to a constant weight. The water content is expressed as the percentage, by weight, of the dry sample. The soap was weighed and recorded as 'wet weight of sample' and was dried from 100 to115^{o}C using a dryer. The sample was cooled down and weighed to find the 'dry weight of sample'. The moisture content was determined using the formula,

% moisture content = (Weight of the wet sample – weight of the dry sample) in g / weight of the dry sample in g * 100.

2.8. Antimicrobial susceptibility testing: The anti-microbial action of the methanolic leaf extract and formulated herbal bath soap on the clinical pathogens from local hospitals such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida sp.* were studied. The micro-organisms were clinical isolates obtained from local hospitals. The culture medium used was Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungus. Well diffusion technique was adopted for antimicrobial susceptibility testing.

3. RESULTS

The yield recovered from 150 g of starter material in soxhlet extraction with methanol as solvent after treating with petroleum ether was 10.75 g (7.17%). The phytochemical analysis of the aqueous leaf extract shows the presence of alkaloids, saponins, tannins, proteins and carbohydrates (Table.1). FTIR spectrophotometer was performed to assess the functional groups of the leaf extract. Fig 1 shows the spectra of functional groups present in *V. negudo* and table 2 shows the functional groups of the possible active constituents can be predicted. The saponification value was found to be 395.52 mg/ml. The range of saponification value for coconut oil is 390 mg/ml which complies with the obtained data. The formulated herbal bath soap using *Vitex negundo* leaf extract is shown in Figures 2(a) and 2(b) with lower and higher concentrations respectively. The physical appearance and other characteristics of the soap were also observed. The soap was totally dried and stable solid bar without any colour change. It is foamy in nature when washed hands even without adding any additional foaming agents such as detergents and surfactants. It also has skin compatibility as it didn't show any irritation when tested on ten users. The pH of the soap was determined as 9.5 with pH strip and 9.67 with pH meter. TFM was estimated to be 70 % and is characterized as grade 2 soap. The moisture content of the soap was found to be 6.23 %. The antibacterial action of the methanolic leaf extract and formulated soaps were studied on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* cultured on MHA. Fig. 3(a) is for the extract and Fig. 3(b) for the soaps. Fig. 3(c) shows the antifungal activity of the extract and soaps on *Candida sp.* cultured on SDA. Well diffusion technique was followed and the diameters of zones of inhibition were measured in mm and the results were tabulated (Tables 3 – 5).

Table.1.Phytochemical Screening analysis of the extract

Chemical constituents with Tests	Presence / Absence
Alkaloids (Mayer's test)	+
Saponins (Foam test)	+
Tannins (Lead Acetate test)	+
Phytosterols (Salkowski test)	+
Flavonoids (Shinoda test)	+
Proteins (Ninhydrin test)	+
Carbohydrates (Molisch's test)	+

+ Present - Absent

Figure.1. FTIR spectra of *Vitex negundo* methanolic leaf extract

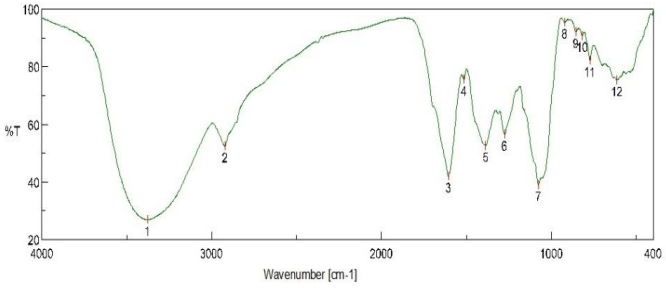

Table 2. Functional group analysis of the extract from FTIR Spectroscopy

No.	Frequency, cm^{-1}	Bond	Functional group
1	3377.71	OH Stretching	Alcohol, phenols
2	2922.59	CH stretching	Alkanes
3	1606.41	C=C Stretching	Alkene
4	1513.85	C=C Stretching	Aromatic
5	1386.57	C-H Bending	Alkanes
6	1274.72	C-O Stretching	Carboxylic acid ester
7	1076.08	C-O-C Stretching	Aliphatic ether
8	919.879	C-O Stretching	Epoxide
9	817.67	C-H Out of plane bending (vibration)	P-substituted phenyl group

Fig. 2 (a) Formulated herbal soap at low concentration (0.25 g) of extract
Fig. 2 (b) Formulated herbal soap at high concentration (0.5 g) of extract

Fig. 2 (a) **Fig. 2 (b)**

Table 3. Antibacterial activity of methanolic *Vitex negundo* leaf extract.

Bacteria	Zones of inhibition (diameter in mm) of samples A to H							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Staphylococcus aureus	9	-	10	11	12	13	13	13
Escherichia coli	13	-	-	-	-	-	-	-
Pseudomonas aeruginosa	22	-	-	-	-	-	-	-

A – Penicillin 0.5 mg/ml – 50 µL; **B** – 10% DMSO - 50 µL; **C** – Extract 0.5 mg/ml - 50 µL; **D** – Extract 1 mg/ml - 50 µL; **E** – Extract 5 mg/ml - 50 µL; **F** – Extract 10 mg/ml - 50 µL; **G** – Extract 50 mg/ml - 50 µL; **H** – Extract 100 mg/ml - 50 µL

Table 4. Antibacterial activity of the formulated herbal bath soap.

Bacteria	Zones of inhibition (diameter in mm) of samples A to C		
	A	**B**	**C**
Staphylococcus aureus	9	11	13
Escherichia coli	9	11	13
Pseudomonas aeruginosa	10	11	13

A – Commercial antibacterial soap 100 mg/ml – 50 µL; **B** – Formulated soap with low concentration extract 100 mg/ml – 50 µL; **C** – Formulated soap with high concentration extract 100 mg/ml – 50 µL

Table 5. Antifungal activity of the extract and soap.

Non-filamentous fungus	Zones of inhibition (diameter in mm) of samples A to G						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Candida sp.	9	-	17	28	32	-	24

A – Ketaconozole 0.5 mg/ml – 50 µL; **B** –10% DMSO 50 µL; **C** – Extract 100 mg/ml - 50 µL; **D** – Formulated soap with low concentration extract 100 mg/ml – 50 µL**; E** – Soap with high concentration extract 100 mg/ml – 50 µL**; F** – Water - 50 µL; **G** – Commercial antifungal soap 100 mg/ml – 50 µL

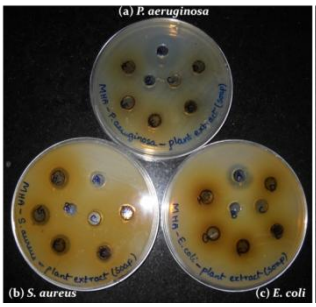

Fig. 3 (a)

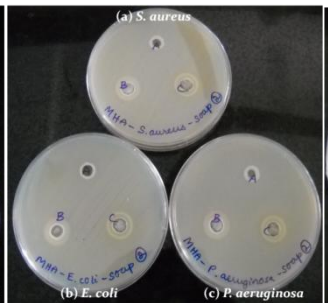

Fig. 3 (b)

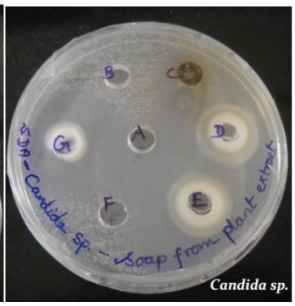

Fig. 3 (c)

Fig. 3 (a) Antibacterial activity of *Vitex negundo* methanolic leaf extract

A – Penicillin 0.5 mg/ml – 50 µL; **B** – 10% DMSO - 50 µL; **C** – Extract 0.5 mg/ml - 50 µL; **D** – Extract 1 mg/ml - 50 µL; **E** – Extract 5 mg/ml - 50 µL; **F** – Extract 10 mg/ml - 50 µL; **G** – Extract 50 mg/ml - 50 µL; **H** – Extract 100 mg/ml - 50 µL

Fig. 3 (b) Antibacterial activity of formulated herbal soap

A – Commercial antibacterial soap 100 mg/ml – 50 µL; **B** – Formulated soap with low concentration extract 100 mg/ml – 50 µL; **C** – Formulated soap with high concentration extract 100 mg/ml – 50 µL

Fig. 3 (c) Antifungal activity of the extract and soap

A – Ketaconozole 0.5 mg/ml – 50 µL; **B** –10% DMSO 50 µL; **C** – Extract 100 mg/ml - 50 µL; **D** – Formulated soap with low concentration extract 100 mg/ml – 50 µL**; E** – Soap with high concentration extract 100 mg/ml – 50 µL**; F** – Water - 50 µL; **G** – Commercial antifungal soap 100 mg/ml – 50 µL

4. DISCUSSION

The present work is a novel herbal soap formulation using *V. negundo* methanolic leaf extract. The major phytochemical constituents with medicinal values have been proved to be present in the methanolic leaf extract from the preliminary phytochemical analyses. The commercial herbal bath soap of choice has 0.38 g of herbal extract in a 75 g soap bar. This was taken as a standard reference and the soaps were formulated with lower (0.25 g of *V. negundo* extract for 75 g soap) and higher (0.5 g of *V. negundo* extract for 75 g soap) concentrations to prove their efficacy even at a concentration less than that of the commercial soaps. From the results obtained, the antibacterial activity of methanolic leaf extract on gram positive *S.aureus* was found to be efficient when compared with gram negative *E. coli* and *P. aeruginosa* which had no visible effect. The anti-fungal activity of the extract and soaps was also studied for a non-filamentous fungus *Candida sp.* Thus, the formulated soaps have shown good antibacterial and antifungal activities when compared with the commercial soaps. The

extract was found to be efficient for fungal cultures rather than bacteria at same concentration, and so with the soap formulations. Hence, the formulated soap was found to be efficient with anti-fungal properties when compared with the commercial soaps which can further be used as a biopharmaceutical product in the treatment for fungal skin infections as well, along with its usage as normal herbal bath soap.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Science and Technology, New Delhi, Govt. of India sponsored National Facility for Drug Development for Academia, Pharmaceutical and Allied Industries (VI-D&P/349/10-11/TDT), Bharathidasan Institute of Technology, Anna University, Tiruchirappalli for its valuable and sophisticated facilities to carry out the present study.

REFERENCES

Alade PI, Irobi ON, Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. Journal of Ethnopharmacology, 39(3), 1993, 171-174.

Alam MI, Gomes A, Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. Journal of Ethnopharmacology, 86(1), 2003, 75-80.

Ayo RG, Amupitan JO, Zhao Y, Cytotoxicity and antimicrobial studies of 1, 6, 8-trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of Cassia nigricans Vahl. African Journal of Biotechnology, 6(11), 2007.

Batubara I, Mitsunaga T, Ohashi H, Screening anti-acne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. Journal of Wood Science, 55(3), 2009, 230-235.

Benjamin TV, Lamikanra A, Investigation of Cassia alata, a plant used in Nigeria in the treatment of skin diseases. Pharmaceutical Biology, 19(2-3), 1981, 93-96.

Biswas TK, Mukherjee B, Plant medicines of Indian origin for wound healing activity: a review. The international journal of lower extremity wounds, 2(1), 2003, 25-39.

Chaudhuri RK, Emblica cascading antioxidant: a novel natural skin care ingredient. Skin Pharmacology and Physiology, 15(5), 2002, 374-380.

Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD, Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. Journal of Ethnopharmacology, 87(2), 2003, 199-206.

Gray AM, Flatt PR, Insulin-secreting activity of the traditional antidiabetic plant Viscum album (mistletoe). Journal of Endocrinology, 160(3), 1999, 409-414.

Guarrera PM, Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. Journal of Ethnopharmacology, 68(1), 1999, 183-192.

Kareru PG, Keriko JM, Kenji GM, Thiong'o GT, Gachanja AN, Mukiira HN, Antimicrobial activities of skincare preparations from plant extracts. African Journal of Traditional, Complementary and Alternative Medicines, 7(3), 2010.

Karunamoorthi K, Ramanujam S, Rathinasamy R, Evaluation of leaf extracts of Vitex negundo L.(Family: Verbenaceae) against larvae of Culex tritaeniorhynchus and repellent activity on adult vector mosquitoes. Parasitology research, 103(3), 2008, 545-550.

Marwah RG, Fatope MO, Mahrooqi RA, Varma GB, Abadi HA, Al-Burtamani SKS, Antioxidant capacity of some edible and wound healing plants in Oman. Food chemistry, 101(2), 2007, 465-470.

Perumal Samy R, Ignacimuthu S, Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. Journal of Ethnopharmacology, 69(1), 2000, 63-71.

Riehemann K, Behnke B, Schulze-Osthoff K, Plant extracts from stinging nettle Urtica dioica, an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-κB. FEBS letters, 442(1), 1999, 89-94.

Saikia AP, Ryakala VK, Sharma P, Goswami P, Bora U, Ethnobotany of medicinal plants used by Assamese people for various skin ailments and cosmetics. Journal of Ethnopharmacology, 106(2), 2006, 149-157.

Sathiamoorthy B, Gupta P, Kumar M, Chaturvedi AK, Shukla PK, Maurya R, New antifungal flavonoid glycoside from Vitex negundo. Bioorganic & medicinal chemistry letters, 17(1), 2007, 239-242.

Shah AJ, Ahmad M, Shaheen F, Antispasmodic effect of Acorus calamus Linn. is mediated through calcium channel blockade. Phytotherapy research, 20(12), 2006, 1080-1084.

Solanki R, Treatment of skin diseases through medicinal plants in different regions of the world. International Journal of Biomedical Research, 2(1), 2011, 73-88.

Telang RS, Chatterjee S, Varshneya C, Study on analgesic and anti-inflammatory activities of Vitex negundo Linn. Indian journal of pharmacology, 31(5), 1999, 363.

Tiwari OP, Tripathi YB, Antioxidant properties of different fractions of Vitex negundo Linn. Food Chemistry, 100(3), 2007, 1170-1176.

Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M, Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat. Therapie, 55(3), 1999, 379-382.