Research Article

Screening of phytochemicals and Antimicrobial activity of flower extract of *Leucas lavandulaefolia* Rees. (Labiatae)

Muthiah Maridass

FISSD'S- Research Institute of Conservation Ecology (Run by Foundation for Innovative Science and Socio -Economic Development), Tirunelveli, Tamilnadu-627011,India.
Email: drmaridass@gmail.com; Mobile : +91-9487567793

Abstract

Background:
The entire plant of *Leucas lavandulaefolia* Rees has been traditionally utilized in Siddha and Ayurveda for addressing various ailments, including psoriasis, persistent skin eruptions, and painful swellings. This research study aimed to assess the antimicrobial properties of the *L. lavandulaefolia* flower and to analyze the phytochemical composition of the plant extract.

Methods:
The fresh flowers of Leucas lavandulaefolia were successively extracted using double-distilled water. The extract's active compounds were screened following standard protocols. The agar well diffusion method was assessed for antibacterial and antifungal activity against human pathogens. The antifungal activities of the extracts were compared with clotrimazole as the positive control and dimethyl sulfoxide (DMSO) as the negative control.

Results:
The antimicrobial activity of the aqueous extract of *L. lavandulaefolia* flower was found to be the highest against *Salmonella typhi* and *S. aureus* (14±0.43mm) and the fungus *C. albicans* (14±0.15mm), with the lowest activity observed against *B. subtilis* (12.9±0.12 mm). The aqueous extract of *L. lavandulaefolia* Rees flowers demonstrated the presence of alkaloids, phenolic compounds, anthocyanins, and essential oils. Furthermore, the isolated active components from the alkaloid residue exhibited the highest levels of antibacterial and antifungal activity.

Conclusions: The aqueous extract of *L. lavandulaefolia* flowers demonstrated in vitro antibacterial and antifungal activities against *Salmonella typhi, S. aureus, C. albicans*, and *A. niger*. In the present study, active compounds such as flavonoids, alkaloids, and anthocyanins may act as antimicrobial agents.

Keywords :*Leucas lavandulaefolia* Rees. (Labiatae), herb, extract, medicinal plants, antimicrobial activity

Cite this Article : Maridass, M. (2025). Screening of phytochemicals and Antimicrobial activity of flower extract of *Leucas lavandulaefolia* Rees. (Labiatae). *Nature of Pharmaceutical Technology*,Vol.No.15(1)pp.1-5.

Journal Information

Journal : Nature of Pharmaceutical Technology (ISSN : 2231– 0436 (Print)

Publisher :Gayathri Global Academic & Scientific Publisher (GGASP), Tirunelveli, Tamilnadu,India

Article History

Article received dated on : 07-11-2024

Corrected and Resubmission dated on 15–03-2025

Published Online : 15-03-2025

1. Introduction

The genus Leucas is among the 250 genera of the family Lamiaceae, distributed throughout the earth's tropical regions. Its immediate ancestor is not known, and the phylogenetic. *Leucas lavandulaefolia Rees* is an herbaceous, highly branched, erect or diffuse annual plant, reaching heights of 30 to 60 cm. It can be found widely throughout India as a weed in cultivated fields, wastelands, and along roadsides. (Anonymous, 1962). The leaves of this common weed are squeezed and the juice that is obtained is placed on wounds to obtain healing (Fox, 1952). This herb is also used in psoriasis, chronic skin eruptions and painful swellings (Anonymous, 1962; Kirtikar and Basu, 1975; Satyabati et al., 1987). Plants have been extensively used by rural people of Mithila region (Bihar) in human and cattle ailments, such as cough, cold, fever, loss of appetite, skin diseases, headache, snake bite and scorpion sting (Chopra and Handa, 1958). The juice of the plant along with a little amount of salt is applied into the eye to cure conjunctivitis. A decoction of leaves is used as a sedative in nervous disorders, as expectorant, carminative, vermifuge and stomachic. Flowers are stimulant, expectorant and diaphoretic. Juice of flower with honey and a few grains of borax mixed together are very much useful for nasal and laryngeal coughs and colds. The aerial parts have a strong characteristic odour and are used as sedative, laxative, anthelmintic, inflammation, jaundice, dyspepsia, vermifuge, stomachic, scabies, psoriasis, dermatosis, migraine, glaucoma, asthma, anthelmintic, urinary discharge, fever and paralysis (Anonymous, 1962; Kirtikar and Basu, 1975; Nadkarni, 1982; Kamat and Singh, 1994). This herb is cooked as vegetable by local tribes in Orissa (Girach and Aminuddin, 1992). It was also reported that after grinding 3 pieces of root, flower of this herb with three teaspoonfuls of turmeric powder with a little bit of water may be given orally to the patient suffering from stomach pain and remarkable cure has been observed (Boissaya and Mazumder, 1980). Literature review of this plants is found in the active compound such as acacetin, chrysoeriol, luteolin, acacetin *7-O-β-D*-glucuronide, acetoside, isoacetoside, salicylic acid and caffeic acid, luteolin, acacetin 7-O-βD-glucuronide, acetoside, isoacetoside, and salicylic acid (Parvin Begum *et al*.,2015). Researchers have reported the different pharmacological activities of this is healing of wound in animal model (Saha *et al*.,1997), hepatoprotective (Chandrashekar and Prasanna, ,2007a; 2010a), hypoglycemic (Chandrashekar and Prasanna, 2010b), Anti-inflammation, analgesic and antipyretic (Saha et al., 1997b, Chandrashekar and Prasanna, 2010c, Mukherjee *et al*., 2002), psychopharmacological (Mukherjee *et al*., 2002b), antidiarrhoel (Mukherjee et al., 1998) ,antiulcer (Gupta et al., 2010) ,antibacterial activity (Saha et al.,1995), hypoglycaemic activity (Saha *et al*.,1997). The aim of the present study was to evaluate the antimicrobial activity of *L. lavandulaefolia* flower and to determine the phytochemical content of the plant extract.

2. Materials and Methods

2.1 Plant Materials

The plant materials of *Leucas lavandulaefolia* Rees flowers collected from the herb in full blossom from waste land of Rajagopalapuram, Tirunelveli District, Tamilnadu, India. Voucher specimen (RICE-Aus2023101) was deposited by our laboratory (FISSD RICE).

2.2 Extraction

100gm of fresh plant materials of flower were added with 250 ml double distilled water and crushed in mortar and pestle. Finally, filtered and collected the extract in round bottom flask. This aqueous extract was then concentrated and dried under reduced pressure. The semi-solid extract was obtained and used for the further experiment. The extraction yield of selected plants has been calculated by the following equation (Felhi *et al*., 2017):

$$\text{Yield } (\%) = (X_1 * 100)/X^0$$

Where X_1 refers to the weight of extract after evaporation of solvent and X^0 refers to the dry weight of the plant powder before extraction

2.3 Isolation of alkaloids and identification

50g of dried and pulverized flowers were extracted with 250 ml methanol in Soxhlet apparatus and the excess of solvent was evaporated in to dryness in vacuo. The methanolic extract (50 mg) was collected and placed in a small test tube, and 1*N* HCl (1mL) was added. The mixture was stirred with a glass rod for 10min in order to achieved complete dissolution of the alkaloids, which, due to their basic character, pass to the acid aqueous solution as salts. A clear extract usually was obtained after filtration and centrifugation. To the solid residue 1*N* HCl (0.5 mL) is added, and the operation is repeated. The combined filtrates (1.5 mL) are divided into three portions, and each was placed into a small test tube. Then, two or three drops of the precipitation reagents-Mayer, Bouchardat, and Dragendorff are added. Positive results are indicated by the formation of a coloured precipitate white (Mayer) or orange (Dragendorff), and brown (Houchardat). Results are registered as abundant (+++), moderate (++), scarce (+), and negative (-). Precipitates can also be formed by proteins, purines, coumarins, and some polyphenols. Because a negative test is indicative of the absence of alkaloids, these reagents are used like presumptive tests for their presence.

2.4 Estimation of total flavonoid content, total phenolic and anthocyanin content

The total phenolic content was measured by using Folin-Ciocalteu colorimetric technique previously described by Haq *et al*.,(2012) and expressed using gallic acid equivalents (mg gallic acid/g of extract). Total flavonoid content of seeds was determined by Colorimetry with aluminum chloride (Chang *et al*.,2002) and expressed using quercetin equivalents (mg of quercetin/g of extract). Anthocyanin content was determined by according to Wegdan Ali Shehata, (2020) method.

2.5 Thin Layer Chromatography (TLC)

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, Germany) to determine the number of compounds present in the flower extract. A total of 5µL of water extract was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Different solvents at various combinations and concentrations (Table-1) were used for metabolite profiling. Development of the chromatogram was carried out in closed tanks by using methanol as the mobile phase, in which the atmosphere was saturated with eluent vapor by wetting a filter paper lining. The chromatogram was visualized under UV light (365 nm and 254 nm) and iodine vapor. The *Rf* values of the compounds were calculated using the following formula.

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

2.6 Antimicrobial activity

Tested pathogens: Bacteria: *Bacillus subtilis, Salmonella typhi, Streptococcus pneumoniae and Staphylococcus aureus* fungi: *Candida albicans,* and *Aspergillus niger*

2.6.1 Preparation of Inoculum

The antimicrobial properties of plant extracts were tested against tested bacteria and fungus such as *Bacillus subtilis, Salmonella typhi, Streptococcus pneumoniae* and *Staphylococcus aureus, Candida albicans,* and *Aspergillus niger*. The tested bacteria were pre-cultured in Mueller Hinton broth (MHB) overnight in a rotary shaker at 37°C. Afterward, each strain was adjusted at a concentration of 10^8 cells/ml using 0.5 McFarland standard (Bhalodia and Shukla, 2011). The fungal inoculum was prepared from the 48h culture of fungal isolates in Potato dextrose broth (PDB) (Nisha *et al*., 2010). The spectrophotometer (A595 nm) has been used to adjust the spore density of the fungus to a final concentration of 10^6 spores/ml.

2.6.2 Antibacterial activity

Antibacterial evaluation of flower extract was done with disc diffusion methods active against *Bacillus subtilis, Salmonella typhi, Streptococcus pneumoniae* and *Staphylococcus aureus, Candida albicans* and *Aspergillus niger*. The tested bacteria were pre-cultured in Mueller Hinton Broth (MHB) overnight at room temperature 36°C. All the tested bacteria (Table-4) were grown in MHB liquid media at 35°C for 12hr, and then 1×10^6 colony-forming units/mL cells were plated on fresh Mueller Hinton Agar (MHA) solid media containing different concentrations of silver nanoparticles (2µg/mL, 4µg/mL, 6µg/mL, and 8µg/mL) incubated at 35°C for 12hr. Control as a blank disc.

2.6.3 Antifungal activity

Fungus of *Candida albicans* and *Aspergillus niger* were grown in Mullar Hinton Broth (MHB) liquid medium at 35°C for 2 days, and then 1×10^6 colony-forming units/mL cells were plated on fresh PDA solid media containing different concentrations of extract (25 mg/mL, 50mg/mL, 75 mg/mL and 100 mg/mL) incubated at 35°C for 5 days. Extract free PDA plates, cultured under the same conditions, were used as controls.

2.7 Data Analysis

The determining antimicrobial activity is based on the inhibition zone diameter measured by inhibition zone diameter >20 mm was categorized as very strong activity, 15-20 mm as strong, 10-14 mm as moderate and 9-7 mm as weak, while <7 mm as no antibacterial and antifungal activities.

3. Results and Discussion

3.1 Preliminary phytochemicals

The results of the present study were observed by the aqueous extract of *L. lavandulaefolia* Rees flower revealed the presence of alkaloids, phenolic compound, anthocyanin and essential oils. The determined the active compounds content of phenolic compounds, flavonoids and anthocyanins were represented in the table-1. Previous studies, Geethika and Kumar (2017) reported the methanolic leaf extract of *L. zeylanica* was found to possess a high amount of phenolics and a minimum quantity of tannins.

Table-1: Preliminary phytochemical analysis of aqueous extract of *Leucas lavandulaefolia* Rees flower

Sl.No	Active constituents	Present/Absent
1	Alkaloids	+++) High)
2	Flavonoids	++ (High)
3	Phenolic Compounds	+++(High)
4	Essential Oils	+++(High)
6	Tannins	+++ (High)
7	Saponins	+++ (High)

3.2 Flavonoids,phenolic and anthocyanin content

The aqueous extract of *L. lavandulaefolia* Rees flower calorimetrically determined by the standard methods and results are represented in the table-2. Previous studies, according to Aryal *et al*., (2019) reported that flavonoid content in maximum level flavonoids was methanol extracts of eight wild vegetables species of *Alternanthera sessilis*, *Basella alba*, *Cassia tora*, *Digera muricata*, *Ipomoea aquatica*, *Leucas cephalotes*, *Portulaca oleracea* and *Solanum nigrum.* Wegdan Ali Shehata *et al*., (2020) reported that anthocyanin content of ethanolic extracts from the dried plants material of apple peel, blueberry and plum peel lesser than the *L. lavandulaefolia*. The relationships between phenolic content of medicinal plants and antioxidant activity are well documented earlier studies (Velioglu *et al*.,1998; Kahkonen *et al*.,1999).

Table-2 : Quantification of active compounds of aqueous extract of *L. lavandulaefolia* flower

Sl.No	Active compounds	Quantification mg/gms
1	Flavonoids (mg of quercetin/g of extract)	12.41 ± 0.12
2	Total phenolics (mg of gallic acid/g of extract)	25.32 ± 1.36
3	Anthocyanin (as cyanidin-3-glucoside)	12.65±0.15

Values are means ± SEM (n = 3).

3.4 Alkaloids spot identification of TLC

Now more than 3000 of alkaloids are known in over different 4000 plant species. Alkaloids in pure form are usually colorless, odorless crystalline solids, but sometimes they can be yellowish liquids. Quite often, they have bitter taste (Kurek Joanna, 2019). The present study was both water and methanolic extracts packed with column chromatography and elution different polarity of solvents and results were represented in the table-3. Previous studies, both alkaloids of asperphenamate and nicotine have been reported in *Leucas aspera (*Mangathayaru *et al*.,2006), Long-chain compounds nonatriacontane (Mishra *et al*.,1995), 1-dotriacontanol, 1- hydroxytetratriacontan-4-one, 32-methyltetratriacontane were reported in *Leucas aspera (Misra et al.,1992)*. Aliphatic ketols, namely, 28-hydroxypentatriacontan- 7 -one, 7-hydroxy- dotriacontan-2- one, 5-acetoxy-triacontane were isolated from the shoots of *Leucas aspera.* Goudgaon *et al*. (2003) reported that the anti-inflammatory activity of *Leucas aspera* is mainly due to its alkaloidal component.

Table-3: Alkaloids spot identification of TLC Rf Values

Sl.No	Solvents	fractions	TLC Rf Values
1	acetone: methanol(l:l)	5	0.65,0.72;0.82;0.96
2	acetone: methanol(l:2)	7	0.24;0.38;0.42;0.56;0.64;0.33;0.46
3	acetone: methanol(l:2)	3	0.34;0.46;0.48

3.5 Antibacterial activity

The aqueous extract of *Leucas lavandulaefolia* flowers tested for antimicrobial activity, the observed result showed good antibacterial activity (Table-3). The maximum level of activity observed the zone of inhibition active against *Salmonella typhi (*14±0.43mm) and lowest activity against *Bacillus subtilis* (12.9±0.12). Previous studies, chloroform and methanolic extract of leaves of *L. lavandulaefolia* had been found to be effective against *E. coli, S. aureus, B. subtilis and P. aeruginosa* (Saha et al., 1995b). The aqueous extract of *L. lavandulaefolia* flowers, tested for antimicrobial activity, and it was showed good antibacterial activity. The antibacterial activity of aqueous extract was shown to be highest activity against *Salmonella typhi* and *S. aureus* (14±0.43mm).

3.6 Antifungal activity

The results of aqueous extract of *L. lavandulaefolia* flower were more antifungal activity seen in the table-4. The aqueous extract of *L. lavandulaefolia* showed the maximum zone of inhibition against both fungus *C. albicans* (14±0.15mm) and *A. niger* (13±0.13mm). Previous studies, similar results according to Flávia dos Santos Silva et al., (2020) reported that 23 hydroethanolic extracts of plants against the fungi *Candida albicans*. A previous study (**Cruz *et al.* 2007**) evaluated the activity of *Z. joazeiro, Caesalpinia pyramidalis* (valid name: *Poincianella pyramidalis), Bumelia sartorum (*valid name*: Sideroxylon obtusifolium*), and *Hymenaea courbaril*, which are plants popularly known for their treatment of mycoses, against *C. albicans.* Babu et al. (2016) studied on the more antifungal activities of methanol extract of *Leucas zeylanica* leaves active against *Candida albicans* and *Aspergillus flavus*. In conclusion, the present study found that *L. lavandulaefolia* flower had good antifungal activity.

Conclusion

The aqueous extract of *L. lavandulaefolia* Rees flower revealed the presence of alkaloids, phenolic compound, anthocyanin and essential oils. The separated alkaloids residue shows maximum antibacterial and antifungal activities. In addition, in this plant had a variety of secondary metabolites that possibly have antimicrobial activities. Studies on in vivo investigations and isolation of specific antimicrobial compounds from these plants are suggested.

Table-4: Antimicrobial activity of flower extracts of *Leucas lavandulaefolia* Rees. (Labiatae) at different concentration

Sl.No	**Extract (mg/mL)**	**Pathogens**					
		Bacillus subtilis	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
1	25	7.4±0.22	7.6±0.27	7.6±0.27	9.4±0.22	11±0.11	9.5±0.56
2	50	11±0.12	9.4±0.22	9.4±0.22	11±0.21	10±0.17	10±0.21
3	75	12±0.33	9±0.33	10±0.33	11±0.33	12±0.33	11.33±0.33
4	100	12.9±0.12	14±0.43	13±0.12	13±0.21	14±0.15	13±0.13

SD± SE Values are expressed as triplicates

Ethics approval and consent to participate
Ethics approval and consent to participate are not relevant in this case, as our research did not involve animals or human subjects.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no existing conflicts of interest.

4. References

Anonymous, (1962). The Wealth of lndia, Raw Materials, Vol. VI. Publication & Information Directorate, CSIR, New Delhi, p.80.

Fox, R.B. (1952). The *Pinatubo negritos*, their useful plants and material culture: medicinal plants and practices. *Philippines Journal of Sciences*, 81 (34):330.

Kirtikar, K.R. and Basu, B.D.(1975). Compositae. In: E. Blatter, J.F. Caius and K.S. Mhaskar (Eds.), Indian Medicinal Plants. Bishen Singh and Mahendra Pal Singh, Dehradun, p.2016.

Satyabati, G.V. Gupta, A.K. and Tandon, N. (1987). Medicinal Plants of lndia. Vol. I1. Indian Council of Medical Research, New Delhi, pp. 161-165.

Nadkarni, A.C. (1982). Indian Materia Medica. 3rd Edn., Vol. I. Popular Prakashan, Mumbai, India.

Kamat, M. and Singh,T.P. (1994). Preliminary chemical examination of some compounds of genus *Leucas* R. Br. *Geobios*, 21: 31-33.

Girach, R.D. and Aminuddin,P.A. (1992). Some little-known edible plants from Orissa. *J. Econ. Tax. Bot*.,16: 61-68.

Boissaya, C.L. and R. Mazumder, (1980). Some folklore claim of the Brahmaputra valley (Assam). *Ethnomedicine*, 16: 139-145.

Parvin Begum, Yanqing Wang and Bunshi Fugetsu,(2015). Biologically active compounds from *Leucas Lavandulaefolia*. *Int. J. Pharm. Sci. Res*.,6(3): 1013-21.

Saha, K., Mukherjee, P. K., Das, J., Pal, M., and Saha, B. P. (1997). Wound healing activity of Leucas lavandulaefolia Rees. *Journal of Ethnopharmacology*,56(2):139-144.

Saha, K., Mukheijee, P.K., Mandal, S.C., Pal, M. and Saha, B.P. (1995b). Antibacterial activity of *Leucas lavandulaefolia* (Labiatae). *Indian Drugs*,32(8): 402-404.

Saha, K., P.K. Mukharjee, M. Pal and B.P. Saha, 1997. Medicinal properties and chemical constituents of *Leucas lavandulaefolia*: A review. *J. Med. Aromat. Plant Sci*.,19: 1045-1048.

Saha, K., Mukherjee,P.K., Das, J., Mandal,S.C., Pal,M. and Saha, B.P. (1997). Hypoglycaemic activity of *Leucas lavandulaefolia* Rees. in streptozotocin-induced diabetic rats. *Phytother. Res*., 11: 463-466.

Chandrashekar, K.S. and Prasanna,K.S. (2010a). Hepatoprotective activity of *Leucas lavandulaefolia* against carbon tetrachloride-induced hepatic damage in rats. *Int. J. Pharma Sci. Res*., 2:101-103.

Chandrashekar, K.S. and Prasanna,K.S. (2010). Hypoglycemic effect of *Leucas lavandulaefolia* wild in alloxan-induced diabetic rats. *J. Young Pharm*., 1: 326-329.

Chandrashekar, K.S. and Prasanna,K.S. (2010). Anti-inflammatory potential of flavones glycoside from ethanol extract of the aerial parts of the plant *Leucas lavandulaefolia*. *Der. Pharma Chemica*, 2: 21-24.

Mukherjee, K., Saha,B.P. and Mukherjee,P.K. (2002b). Psychopharmacological profiles of *Leucas lavandulaefolia* Rees. *Phytother. Res*.,16: 696-699.

Mukherjee, P.K., Saha,K., Murugesan,T., Mandal, S.C., Pal,M. and Saha,B.P. (1998). Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. *J. Ethnopharmacol*., 60: 85-89.

Gupta, J.K., Upmanyu,N., Patnaik, A.K. and Mazumder,P.M. (2010). Evaluation of anti-ulcer activity of *Leucas lavandulifolia* on mucosal lesion in rat. *Asian J. Pharm. Clin. Res*.,3: 118-118.

Mukherjee, K., Saha,B.P. and Mukherjee,P.K. (2002). Evaluation of antipyretic potential of *Leucas lavandulaefolia* (Labiatae) aerial part extract. *Phytother. Res*.,16:686-688.

Saha, K., Mukherjee,P.K., Das,J., Pal,M. and Saha,B.P. (1997). Wound healing activity of *Leucas lavandulaefolia* Rees. *J. Ethnopharmacol*., 56: 139-144.

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N. and Kadri, A. (2017). Solvent extraction effects on phytochemical constituents' profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *J. Food Sci. Technol*. 37, 483-492.

Haq, N., Ullah, G., Bibi, S., Kanwal, M.S. and Mirza,A.B. (2012). Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iran J. Pharm. Res*.,11: 241-249.

Chang, C.C., Yang, M.H., Wen, H.M., and Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*., 10:178-182.

Wegdan Ali Shehata, M.D., Sohail Akhtar and Tanveer Alam, (2020). Extraction and estimation of anthocyanin content and antioxidant activity of some common Fruits. *Trends in Applied Sciences Research*, 15: 179 -186.

Bhalodia, N. R., and Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: an ethnomedicinal plant. *J. Adv. Pharm. Technol. Res*., 2: 104-109.

Nisha, M.C., Subramanian, M.S., Prathyusha, P., and Santhanakrishnan, R. (2010). Comparative studies on antimicrobial activity of *Artemisia sieversiana* Ehrhart. Ex. Willd. and *Origanum vuigare* L. Int. J. Pharmtech Res. 2, 1124-1127.

Geethika, K. and Kumar, P.S. (2017). Preliminary phytochemical screening of 6 members of *Leucas* (Lamiaceae). *International Journal of Pharmaceutical Sciences Review and Research*, 47(1): 60-64.

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N.(2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants* (Basel),11:8(4):96.

Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D.(1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem*., 46: 4113-4117.

Kahkonen, M.P., Hopia A.I., Vuorela, H.J., Rauha J.P., Pihlaja, K., Kujala, T.S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*., 47: 3954-3962.

Kurek Joanna, (2019). Introductory Chapter: Alkaloids - Their Importance in Nature and for Human Life'. Alkaloids - Their Importance in Nature and Human Life. Intech Open. doi:10.5772/intechopen.85400.

Mangathayaru, K., Thirumurugan, D., Patel, P.S., Pratap, D.V., David, D.J. and Karthikeyan, J. (2006). Isolation and identification of nicotine from *Leucas aspera* (willd) link. *Indian J Pharm Sci.,* 68:88-90.

Mishra, T.N., Singh, R.S., Pandey, H.S. and Singh, S.(1995). A novel phenolic compound from *Leucas aspera*. *Indian J Chem.,* 34:1108-10.

Mishra, T.N., Singh, R.S., Pandey, H.S. and Singh, S.(1992). Long-chain compounds from *Leucas aspera*. *Phytochemistry,* 1992;31:1809-10.

Goudgaon, N.M., Basavaraj, N.R. and Vijayalaxmi, A.(2003). Antiinflammatory activity of different fractions of *Leucas aspera* SPRENG. *Indian J Pharmacol.,*35:397-8.

Flávia dos Santos Silva, Melissa Fontes Landell, Gustavo Vasconcelos Bastos Paulino, Henrique Douglas Melo Coutinho and Ulysses Paulino Albuquerque,(2020). Antifungal activity of selected plant extracts based on an ethnodirected study. *Acta Bot. Bras.,*34 (2):443-445.

Cruz, M.C.S., Santos, P.O., Barbosa, A.M, *et al*.,(2007). Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. *Journal of Ethnopharmacology*, 111: 409-412.

Babu, A., Mohamed, A.S.N., Jaikumar, K., Anand, D. and Saravanan, P. (2016). *In-vitro* antifungal activity of leaf extracts of *Leucas aspera* and *Leucas zeylanica*. *International Journal of Pharmaceutical Sciences and Research*,7(2): 752-756.