Indian J. Applied & Pure Bio. Vol. 39(1), 113-120 (2024). **ISSN: 0970-2091**
A Web of Science Journal

GC-MS analysis, phytochemical screening and Antimicrobial potential of methanolic extract of *Malva parviflora* L. and *Phyllanthus niruri*, L.

***Vijay Pal, [1]Sheetal Tanwar, [2]Aarti Chaudhary, and [3]Rahul**

[*]Department of Botany, Shri ShRaddhanath P.G. College Todi, Gudha Gorji, Jhun Jhunu-333022 (India)
[1]Department of Botany, GGDSD College, Palwal-121 102 (India)
[2]Department of Botany, Raja Balwant Singh College, Dr. Bhimrao Ambedkar University, Agra-282 002 (India)
[3]Department of Botany, Maharshi Dayanand University Rohtak-124001 (India)
*(e-mail: Gujjarvijay66@gmail.com; Mobile: 9466931266)

Abstract

This investigation aimed to examine the antibacterial activity and phytochemical analysis of *Malva parviflora* L. and *Phyllanthus niruri* L. whole plant. The methanolic extract was analyzed by using GC-MS, and the results showed that it contained a variety of phytochemicals like Borane, chlorodipropyl; Propane, 1,2,3 trichloro; 2-Troponyl difluoroborate; 1,4 Benzenedicarboxylic acid; dimethyl ester; Methyl 12,13 tetradecadienoate; 7-Hexadecenoic acid; methyl ester; Methyl 9,10 octadecadienoate; beta-Sitosterol acetate; 1-(10,10-Dimethyl-3,3- dioxo-3-thia-4- azatricyclo[5.2.1.0(1,5)] dec-4-yl)-3-methylpent4-en-1-one; Methyl 8,9- octadecadienoate in *Malva parviflora* whole plant. *Phyllanthus niruri* also shows the phytochemicals like Cyclopentyl-methylphosphinic acid; 2- isopropyl-5-methylcyclohexyl ester; Methyl 9,10- octadecadienoate; 2,3-Bis [(4-hydroxy-3- methoxyphenyl) methyl] butane-1,4-diol, tetramethyl ether; Methyl 11,12- tetradecadienoate; Methyl 12,13- tetradecadienoate. Research has also been done to investigate the antibacterial activity of the methanolic extract against a variety of bacterial and fungal species, when employed in concentrations of 1µL, the methanolic extract of *Malva parviflora* showed a minimum inhibition zone of 8 mm against the bacteria, *Escherichia coli* and 5 mm *Serratia marcenscens*. Similarly utilized in concentrations of 1 µL for antifungal action 7 mm of minimum inhibition zone against *Aspergillus niger* and 5mm against *Penicillium digitatum*. Similarly, *Phyllanthus niruri* shows 5mm of inhibition against *Escherichia coli* and *Serratia marcenscens*. The 5mm of inhibition was

observed against *Aspergillus niger* and 10 mm against *Penicellium digitatum*. These findings imply that *Malva parviflora* and *Phyllanthus niruri* have the potential to be used in pharmaceutical industries, both may work as promising natural antibacterial agents.

Key words : *Malva parviflora, Phyllanthus niruri,* antibacterial, antifungal, phytochemicals, Gas chromatography, and mass spectrometry.

Our ancestors started the history of phytotherapy, who had the knowledge of herbs and knew how they could be the source in resulting the soothing effect against certain kinds of diseases, the practice of using plant based drugs was 5000 year old practice[8] Medicinal plants used to treat many diseases have been an old tradition. The drugs produced by the plants are curative, easily available, safe to use, less expensive, and have rare side effects. *Phyllanthus niruri* is a medicinal plant belonging to the family Euphorbiaceae that is commonly known as' Bhoomi Amla' in Indian language. *Phyllanthus* genus includes over 700 species that are distributed in the tropical and subtropical regions[7]. *Malva parviflora* belongs to the family Malvaceae and fossils have been found 23.03 to 5.333 million years ago[4,15]. There are some reports on the use of Malva spp. in silver nanoparticle biosynthesis[14]. Traditional herbalists and healers used dried powder or an infusion prepared from leaves and roots of *Malva parviflora* for cleaning wounds and sores, for treating wounds and swellings leaves are also used for making a hot poultice as it contains tannin, flavonoids, polyphenols, saponins, resins, and some alkaloids also, for treatment of bruised and broken limbs it is incorporated into a lotion, treatment for mouth and throat infection[11]. *Phyllanthus niruri* is used as a medicinal plant for various diseases like kidney stones, fever, stomach ache, diabetes, jaundice, asthma, gonorrhea, diarrhea, ulcers wounds, bronchial infections, and other genital infections[3,5,9].

Sample preparation: *Phyllanthus niruri* and *Malva parviflora* were collected from the botanical garden and identified by the Botany Department of GGDSD College Palwal Haryana. Plant material was dark dried at room temperature and ground into a fine powder with the help of a mortar and pestle, 10gm powder was mixed in 100ml methanol and left for 24hrs, a solution was filtered and the filtrate was collected and stored at 4° till further studies.

GC-MS Analysis: A gas chromatography coupled with mass spectrometry (GC-MS) is a combined analysis that has superior ability in analyzing organic/small biomolecules qualitatively. *Phyllanthus niruri* and *Malva parviflora* methanolic extract was analyzed by Gas Chromatography–Mass Spectrometry Agilent 8890/ 5977B series Agilent 5977B EI/ CIMSD, pressure ranges 0.001 to 13.886 psi with 0.01 to 100 psi resolution, with 30m× 250µm× 0.25µm front SS inlet with nitrogen as a carrier gas and same in back SS inlet but helium as a carrier gas, which flow 1ml/min. The temperature range was set to 60° - 280° for 50 minutes. The solution was diluted in

methanol 0.1 mg/mL with molecular weight less than 500g/mol, 1µL solution was used for injection in S1inlet *Malva parviflora* and S2 was *Phyllanthus niruri* Initial average velocity at 160° was 38.194 cm/sec and hold up time was 1.30 minute. At the rear SS a triple axis detector with high energy dynode and electron multiplier auto-sampler, temperature was 114.3° and electron mass 236.3Hz which was connected to TIC, MS library (NIST20.L) with Agilent Mass Hunter.

Antimicrobial Assay :

Muller Hinton Agar medium was used for antibacterial activity by disk/well diffusion susceptibility method. Cultured bacterial samplers were mixed with peptone water to make 0.5 Mc Farland Turbidity standards. The culture sample was swabbed on Muller Hinton agar surface and wells were created with the help of sterile tips. Sabouraud Dextrose Agar medium was used for antifungal activity by well diffusion method. Cultures were mixed with peptone water to make 0.5 Mc Farland Turbidity standards. The culture was swabbed on Sabouraud Dextrose Agar surface and wells were created with the help of sterile tips. The methanolic extract was used against *Escherichia coli, Serratia marcenscens* for antibacterial and *Aspergillus niger,* and *Penicellium digitatum* for antifungal. Amoxicillin potassium clavulanate was used as positive control and methanol as a negative control.

GC-MS analysis : The *Malva parviflora* whole plant GC-MS analysis shows the presence of a different type of phytochemicals like Borane, chlorodipropyl ($C_6H_{14}BCl$); Propane, 1,2,3 trichloro ($C_3H_5Cl_3$); 2-Troponyl difluoroborate ($C_7H_5BF_2O_2$); 1,4 Benzenedicarboxylic acid, dimethyl ester ($C_{10}H_{10}O_4$); Methyl 12,13 tetradecadienoate ($C_{15}H_{26}O_2$); 7-Hexadecenoic acid, methyl ester, (Z) ($C_{17}H_{32}O_2$); Methyl 9,10 octadecadienoate ($C_{19}H_{34}O_2$); beta-Sitosterol acetate ($C_{31}H_{52}O_2$); 1-(10,10-Dimethyl-3,3-dioxo-3-thia-4-azatricyclo[5.2.1.0(1,5)] dec-4-yl)-3-methylpent 4-en-1-one ($C_{16}H_{25}NO_3S$); Methyl 8,9- octadecadienoate ($C_{19}H_{34}O_2$). *Phyllanthus niruri* whole plant consists of Cyclopentyl-methylphosphinic acid, 2- isopropyl-5-methylcyclohexyl ester ($C_{16}H_{31}O_2P$); Methyl 9,10- octadecadienoate ($C_{19}H_{34}O_2$); 2,3-Bis[(4-hydroxy-3- methoxyphenyl)methyl] butane-1,4-diol, tetramethyl ether ($C_{24}H_{34}O_6$); Methyl 11,12- tetradecadienoate ($C_{15}H_{26}O_2$); Methyl 12,13- tetradecadienoate ($C_{15}H_{26}O_2$) (Table-1) The presence of beta-Sitosterol acetate phytochemical in *M. parviflora* suitable for antimicrobial potential it is an ingredient of various ancient herbal formulation.

Antimicrobial assay :

Malva parviflora whole plant was studied for antibacterial potential by disc diffusion assay and 1µl of methanolic extract was used against the *Escherichia coli* and *Serratia marcenscens,* a 8mm and 5 mm minimum inhibition zone was observed respectively. *Phyllanthus niruri* extract was also used in the same concentration against the same strains *Escherichia coli* and *Serratia marcenscens* which shows a 5mm inhibition zone. When the *M. parviflora* extract was used against fungal Strains *Aspergillus niger* and *Penicellium digitatum* for antifungal potential the plant shows 7mm of inhibition

Table-1. GC-MS analysis of *Malva parviflora* and *Phyllanthus niruri*

Sr No.	Phytochemical of *Malva parviflora*	Chemical Formula	Phytochemicals of *Phyllanthus niruri*	Chemical Formula
1	Borane, chlorodipropyl	$C_6H_{14}BCl$	Cyclopentyl-methylphosphinic acid, 2- isopropyl-5-methylcyclohexyl ester	$C_{16}H_{31}O_2P$
2	Propane, 1,2,3 trichloro	$C_3H_5Cl_3$	Methyl 9,10-octadecadienoate	$C_{19}H_{34}O_2$
3	2-Troponyl difluoroborate	$C_7H_5BF_2O_2$	2,3-Bis[(4-hydroxy-3-methoxyphenyl) methyl] butane-1,4-diol, tetramethyl ether	$C_{24}H_{34}O_6$
4	1,4 Benzenedicarboxylic acid, dimethyl ester	$C_{10}H_{10}O_4$	Methyl 11,12-tetradecadienoate	$C_{15}H_{26}O_2$
5	Methyl 12,13 tetradecadienoate	$C_{15}H_{26}O_2$	Methyl 12,13-tetradecadienoate	$C_{15}H_{26}O_2$
6	7-Hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	-	-
7	Methyl 9,10 octadecadienoate	$C_{19}H_{34}O_2$	-	-
8	beta-Sitosterol acetate	$C_{31}H_{52}O_2$	-	-
9	1-(10,10-Dimethyl-3,3- dioxo-3-thia-4- azatricyclo[5.2.1.0(1,5)] dec-4-yl)-3-methylpent4-en-1-one	$C_{16}H_{25}NO_3S$	-	-
10	Methyl 8,9- octadecadienoate	$C_{19}H_{34}O_2$	-	-

Table-2. Antimicrobial property of methanolic extract of *Malva parviflora* and *Phyllanthus niruri*

S. No.	Compound	*Escherichia coli*	*Serratia marcescens*	*Penicillium digitatum*	*Aspergillus niger*
1	*Malva parviflora*	8mm	5mm	5mm	7mm
2	*Phyllanthus niruri*	5mm	5mm	10mm	5mm
3	Amoxicillin and potassium clavulanate (positive control)	10mm	10mm	10mm	10mm
4	Methanol (negative control)	00mm	00mm	00mm	00mm

Table-3. Phytochemical present in *Malva parviflora* and *Phyllanthus niruri* methanolic extract

Sr No.	Chemical present	Malva parviflora	Phyllanthus niruri
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Saponins	+	+
4	Steroids	+	+
5	Glycosides	+	+
6	Tannins	+	+
7	Terpenes	+	+

against *Aspergillus niger* and 5mm of inhibition against *Penicellium digitatum.* when a similar type of concentration of *Phyllanthus niruri* was used against the same fungal strain, 5mm of inhibition was observed against *Aspergillus niger* and 10 mm of inhibition was observed against *Penicellium digitatum* (Table-2). The methanol used as negative control did not show any inhibition zone against any bacterial and fungal strain, Amoxicillin potassium clavulanate which is used as a positive control against both plant extract, bacterial and fungal strains shows the 10mm of minimum inhibition zone. The Methanolic extract of the plant *Malva parviflora* aerial parts used against *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus sonnei* shows antibacterial properties[10]. *Malva parviflora* whole plant methanolic and hexane extract shows antibacterial properties against various gram-positive and gram-negative bacteria[1]. According to Shale *et al.*[11] methanolic extract of *Malva parviflora* shows antibacterial potential against gram positive bacteria – *Micrococcus luteus* (ATCC4698), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600) and *Staphylococcus epidermidis* (wild type) and gram-negative bacteria as *E. coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10175), and *Klebsiella pneumoniae* (ATCC13883). These findings are in agreement with our findings which indicate that both plants have antimicrobial potential and can be utilized in herbal formulations against various microbes. The antimicrobial activity of *Phyllanthus niruri* has been observed against fungal and bacterial strains[6]. *Phyllanthus niruri* whole plant extract shows the inhibition against *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhii* when tested by agar well diffusion method report shows that the more the concentration of extract more the inhibition zone[2]. The Aqueous, methanolic and ethanolic extract of *Phyllanthus niruri* were tested for antifungal and antibacterial activities against *Aspergillus niger, Escherichia coli, Bacillus subtilis, Staphylococcus aureus* in which methanolic and ethanolic extract shows the maximum inhibition zone in compare to aqueous extract[13].

Phytochemicals analysis :

Malva parviflora and *Phyllanthus niruri* whole plant show the presence of tannins, alkaloids, flavonoids, glycosides, terpenoids, and saponins (Table-3). **Glycosides:** 4ml extract solution was dried 2ml and 1ml ammonium hydroxide mixed and shaken until a reddish color developed, reporting the presence of glycosides. **Tannins :** 2ml extract was mixed with 2%, 2ml $FeCl_3$ a blue-black color was developed indicating the presence

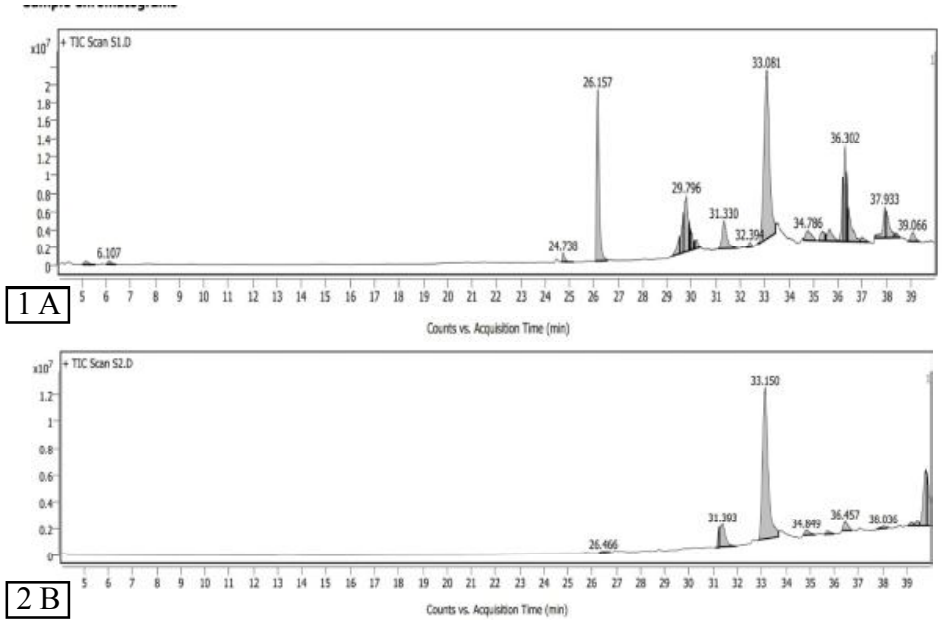

Fig. 1. 1A shows the chromatogram for *Malva Parviflora* and 1B chromatogram of *Phyllanthus niruri*

of tannins. **Steroids:** The plant extract was mixed with 2ml of chloroform and 2ml of sulphuric acid was added by side a red upper and yellow lower layer was produced showing the presence of steroids. **Flavonoids:** 2ml extract was mixed with aqueous NaOH a yellow-orange color developed to indicate the presence of flavonoids. **Saponins:** 2ml extract was mixed with 5ml of distilled water and was shaken vigorously for 30 secs and observed for 30 minutes stable foam was produced indicating the presence of saponins. **Alkaloids:** 2ml extract was mixed with 2ml 1% of HCl and heated, Mayer‘s and Wagner‘s reagent was added and turbidity was developed showing the presence of Alkaloids. **Terepenoids:** 2ml extract was dissolved in 2ml of chloroform and evaporated to dryness, 2ml of concentrated H_2SO_4 was added a reddish brown coloration was formed at the interface indicating the presence of terepenoids (Table-3).

GC-MS analysis of *Malva parviflora* and *Phyllanthus niruri* methanolic extract shows the presence of Borane, chlorodipropyl; Propane, 1,2,3 trichloro; 2-Troponyl difluoroborate; 1,4 Benzenedicarboxylic acid; dimethyl ester; Methyl 12,13 tetradecadienoate; 7-Hexadecenoic acid; methyl ester; Methyl 9,10 octadecadienoate; beta-Sitosterol acetate; 1-(10,10-Dimethyl-3,3-dioxo-3-thia-4- azatricyclo [5.2.1.0(1,5)] dec-4-yl)-3-methylpent4-en-1-one; Methyl 8,9- octadecadienoate in *Malva parviflora*. And Cyclopentyl-methylphosphinic acid; 2- isopropyl-5-methylcyclohexyl ester; Methyl 9,10- octadecadienoate; 2,3-Bis [(4-hydroxy-3- methoxyphenyl) methyl] butane-

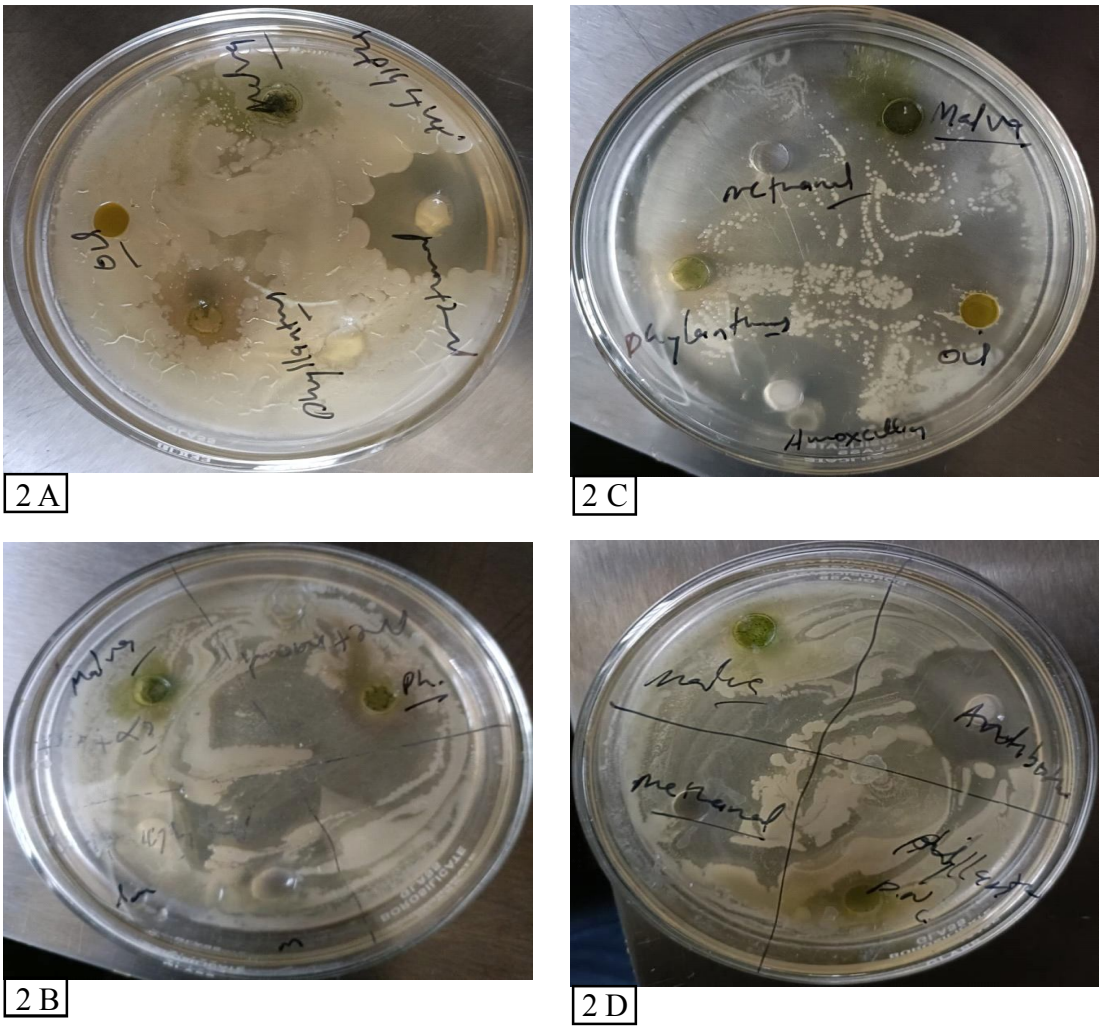

Fig. 2. 2A shows minimum inhibition zone against the *Penicillium digitatum,* 2B *Aspergillus niger*.2C for *Serratia marcescens* and 2D for *Escherichia coli*.

1,4-diol, tetramethyl ether; Methyl 11,12-tetradecadienoate; Methyl 12,13- tetradecadienoate in *Phyllanthus niruri.* Both plants show antimicrobial potential against bacterial strains *Escherichia coli* and *Serratia marcenscens,* and fungal strain *Aspergillus niger* and *Penicellium digitatum.* The above reports show that both plants have a curative potential against various microbes and can be used in herbal formulation.

Statements and Declarations

Competing Interests: The authors of this manuscript have no conflict of interest.

Funding: Not applicable.

Availability of data and materials: Not applicable.

All authors of the manuscript agree to publish this manuscript in Indian Journal of Applied Pure Biology. No matter whether the manuscript has been adopted from previously published papers, so there is no need to get consent from other publishers.

Vijay Pal is thankful to IIT Delhi CRF Synthesis Facility (IIT Delhi) for their support towards the GC-MS facility.

Author's Contributions: Sheetal Tanwar: collected the sample and prepared it for study, Vijay Pal: Conducted the experiments, collected data and wrote the major part of the manuscript, conceptualized the idea, and proof read. Aarti: contributed to writing the manuscript. Rahul: contributed to writing the manuscript.

References :

1. Akbar S, U Hanif, J Ali, and S. Ishtiaq (2014). *Asian Pac j of trop biomed. 4*(5): 410-415. https://doi.org/10.12980/APJTB.4.2014C1107
2. Awache I, I Chinedu, MN Yakubu, A Ubandoma, M Gero, and MN. Ononimu (2017). *Phyllantus niruri* (Chanca Piedra) extracts on some enteric. World.; *2*(6): 39-45.
3. Bello I, NS Usman, A Dewa, K Abubakar, N Aminu, MZ Asmawi, and R Mahmud (2020) *J ethnopharmacology. 250:* 112461. https://doi.org/10.1016/j.jep.2019.112461
4. Boulos L. (2000). Flora of Egypt Cairo. AI Hadara Publishing, *2:* 93-95.
5. Hassim N, M Markom, MI Rosli, and S Harun (2019) *Chem Eng Process. 139:* 14-22. https://doi.org/10.1016/j.cep.2019.03.011
6. Hoffman BR, AH Delas, K Blanco, N Wiederhold, RE Lewis, and L. William (2004). *Pharmaceut Biol.* 42. https://doi.org/10.1080/13880200490504925
7. Jones K, and Pau d'Arco. (1995) Immune power from the rain forest. Inner Traditions/ Bear & Co.
8. Pal V, R Chug, KS Verma, P Sharma, and VS. Gour (2022). Phytomedicines for Bacterial Infections. *Current Developments in the Detection and Control of Multi Drug Resistance. 26:* 79.
9. Patel JR, P Tripathi, V Sharma, NS Chauhan, and VK. Dixit (2011). *J ethnopharmacol 138*(2): https://doi.org/10.1016/j.jep.2011.09.040
10. Shadid KA, AK Shakya, RR Naik, N Jaradat, HS Farah, N Shalan, and GA. Oriquat (2021). J Chem. ; https://doi.org/10.1155/2021/8867400
11. Shale TL, WA Stirk, and J. van Staden (1999). *J of Ethnopharmacol., 67*(3): 347-354. https://doi.org/10.1016/S0378-8741(99)00035-5
12. Shale TL, WA Stirk, and J. Van Staden (2005) *J Ethnopharmacol. 96*(1-2): 325-330. https://doi.org/10.1016/j.jep.2004.09.032
13. Shilpa VP, K Muddukrishnaiah, BS Thavamani, V Dhanapal, KN Arathi, KR Vinod, and SR Sreeranjini (2018) *Environ Diseas. 3*(3): 63.
14. Zayed MF, WH Eisa, and A A. Shabaka (2012). *Spectrochimica Acta Part A: Mol Biomol Spectr. 98:* 423-428. https://doi.org/10.1016/j.saa.2012.08.072
15. Zohary M. (1987). Flora palaestina. *The Israel Academy of sciences and humanities part II:* pp 315-319.