Phytochemical and *In Vitro* Biological Evaluation of Roots of *Malvastrum coromandelianum* (L.) Garcke

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Summary: *Malvastrum coromandelianum* (L.) Garcke is a medicinal plant, employed traditionally for the treatment of different human illnesses. This study has been planned to study the chemical and biological aspects of the roots of the plant, using chemical and instrumental analytical techniques. Standard reported protocols were used for phytochemical screening of the crude extract, indicating the presence of terpenoids, steroids, flavonoids, and alkaloids classes of compounds. The extract was analyzed through Gas Chromatography-Mass Spectrometry (GC-MS) to confirm the presence of specific phytochemicals. The extract exhibited biological activities at good to moderate levels and was found to be non-toxic. Moderate inhibitory potential was observed against *Aspergillus niger, Fusarium solani, Candida albicans, Klebsiella pneumoniae, Bacillus subtilis, Proteus mirabilis, Escherichia coli,* and *Staphylococcus aureus*. At a concentration of 1000 μg/ml the crude extract showed 67 % inhibition of the *Leishmania tropica* promastigotes. The extract also displayed moderate radical scavenging activity. Interestingly, it showed lower red blood cell hemolysis (28%) at the highest concentration. The biological potential of the crude extract may be credited to these metabolites, as most of them have been previously reported to have the same activities.

Keywords: *Malvastrum coromandelianum*, Hemolysis, Anti-fungal, Anti-bacterial, Leishmanicidal, GC-MS analysis, Anti-cancer.

Introduction

Plants, herbs, animals, and microbes are nature's laboratories for the discovery of pharmacologically important chemicals [1], used originally or modified into pharmaceutical agents for the treatment of human ailments [2].

M. coromandelianum L. Garcke (Malvaceae), a three-lobed false mallow [3], is native to the Americas, Australia, Africa, and Asia [4]. Ethnobotanical survey has revealed that *M. coromandelianum* possesses anti-nociceptive, anti-inflammatory, analgesic, anti-cancer, antioxidant, anthelmintic, hypoglycemic, anti-fungal, and anti-bacterial properties [5-13]. Different parts of the plant are being used to cure ringworm infection, jaundice, and diabetes by tribal populations throughout the world [14, 15]. Some bioactive isolates have also been reported from the leaves and stem of the plant [16, 17]. Literature survey thus infers significant pharmacological potential of the aerial parts of the plant, however no detailed information on its phytochemical and pharmacognostic aspects has been reported thus far. Moreover, the underground parts of the plant are yet to be studied in detail. This research project was designed to establish the chemical composition, anti-microbial, Leishmanicidal, anti-cancer, and hemolytic activity of the roots of *M. coromandelianum.*

Experimental

Plant material

Fresh roots of *M. coromandelianum* (3 Kg) were collected in August (2020) from District Swat (Kabal), Khyber Pakhtunkhwa, Pakistan. The plant material was identified at the Department of Botany, University of Peshawar, by Prof. Dr. Siraj-ul-Haq. A voucher specimen (Bot 17315 PUP) was deposited in the herbarium of the same department. The roots were shade dried and ground to powder.

Extraction

Powdered (1 Kg) material was macerated with 50% methanol-water (10 l) and kept for a few days at room temperature. The extract was filtered and the solvent evaporated *in vacuo* to obtain yellowish gummy crude extract (60 g).

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Qualitative phytochemical screening

The presence of phytochemicals *viz*, proteins, saponins, terpenoids, carbohydrates, alkaloids, steroids, flavonoids, cardiac glycosides, reducing sugars, and phlobataninns was determined by following standard procedures, as given below [18, 19].

Test for alkaloids

Aqueous solution of the crude extract (2 ml) was supplemented with 0.5 ml of 1% HCl, followed by addition of Wagner's reagent to the mixture. Reddish-brown appearance indicates the presence of alkaloids.

Test for saponins

A few drops of water were added to the crude extract and stirred to see if there is any foam formation, which would be an indication of the presence of saponins.

Test for terpenoids

To 1 ml sample of extract, 2 ml of chloroform was added, followed by addition of 3 ml of conc. sulfuric acid. Appearance of red brown color at upper layer shows the presence of terpenoids.

Test for carbohydrates

A mixture of the plant extract and 1 ml of Barfoed's reagent ($CuSO_4$) was heated in a water bath at high temperature for 2-3 minutes. A change in color or formation of precipitates shows the presence of carbohydrates.

Test for proteins/amino acids

A mixture of the crude extract (1 ml) and ninhydrin solution (2 ml) was boiled. Appearance of violet color indicates the presence of amino acids.

Test for steroids

The plant extract (1 ml) was dissolved in chloroform (10 ml), followed by the addition of an equal volume of conc. sulfuric acid. The top layer in the test tube turns red, indicating the presence of steroids.

Test for flavonoids

To a 2 ml solution of the extract, 5 ml of H_2SO_4 and 0.5 mg of Mg-ribbon were added. Appearance of a reddish-brown color indicates the presence of flavonoids.

Test for glycosides

The extract was hydrolyzed and neutralized with conc. hydrochloric acid. Appearance of red precipitate confirms the presence of glycosides.

GC-MS analysis

The methanolic extract was analyzed on a Thermo Fisher Scientific DSQII instrument, equipped with a capillary column (30 m long with 0.25 mm internal diameter and thickness). The sample was initially treated with sodium sulphate and filtered through a Whatman filter paper No. 1 to remove any suspended solids and traces of water, followed by filtration. The injector was operated in split mode with a 1 ml/minute flow rate. The pre-treated sample (1 μl) was injected at 50 ^oC and held for 2 minutes until gradually increasing the temperature to 150 ^oC at 8 ^oC/min and then to 300^o C @ 15 ^oC/min, where it was held for 5 minutes. The chemical components were identified from a built-in NIST library.

Biological activities

Anti-fungal activity

Potato dextrose agar (PDA) (7.9 g) in distilled water (200 ml), was used to prepare media for fungal culture. The media was sterilized in an autoclave. The sterilized media was transferred to petri dishes inside a laminar flow cabinet and inoculated with fungal spores after solidification. Fluconazole was used as the positive, while DMSO was the negative control. Wells of appropriate size were punctured in the media and the test sample was administered to the wells at 30, 50, and 100 μg/ml concentrations. The dishes were incubated for 72 hours at 37 ^oC and results recorded thereafter [20].

Anti-bacterial Assay

Anti-bacterial activity was performed using the disc diffusion method. Nutrient agar (8.4 g) was dissolved in 200 ml distilled water to prepare the media. The media was sterilized at 121 ℃ for 15 min. The sterilized media was transferred to petri dishes and inoculated with bacterial spores after solidification [21]. Three concentrations (30, 50, and 100 μg/ml), were prepared from the test sample in DMSO. Ofloxacin disc (5 μg/ml) was used as a positive control. Results were recorded after incubation of the petri dishes for 24 hours at 37 ^oC [22].

Hemolytic Activity

The hemolytic assay was executed as per the procedure followed by Mehwish et. al. [23]. A trained health expert took fresh human blood from participants. All ethical formalities were fulfilled as per the 1975 Helsinki Declaration (revised 1997). Red Blood Cells (RBCs) were separated from the solution by centrifugation (1000× g for 10 min). RBCs were then treated with the test sample. The final concentration was set to be 1000, 500, and 250 μg/ml. All the samples were incubated at 37 ºC for 3 hours. Hemoglobin was released in the supernatant. Negative (DMSO) and positive (0.5% Triton X-100) controls were also run alongside the test samples. The results were recorded on an ELISA plate reader at 576 nm.

Percent hemolysis was measured using the following equation.

$$\%\ hemolysis\ = \frac{mean\ OD\ of\ sample - \ mean\ OD\ of\ blank}{mean\ OD\ of\ positive\ control - mean\ OD\ of\ blank}\ x\ 100$$

Leishmanicidal assay

The *Leishmania tropica* promastigote (1 x 10^4 cells/well) were grown in 96-well microtiter plates in FBS (Gibco®) (10%) supplemented RPMI-1640 (Gibco®) and antibiotics (1%). The promastigotes were allowed to grow for 72 hours at 25 °C in the presence of 1000, 500, and 250 μg/ml of the crude methanolic extract of *M. coromandelianum* [24]. After incubation, the promastigote viability was calculated by the MTT (3-(4,5-dimethylthiazol- 2yl)-2,5-diphenyltetrazolium bromide) colorimetric technique [25]. To start with, MTT dye (100 μl) was applied to the wells, which were incubated for 3 hours at 37 °C. DMSO (40 μl) was used as a solvent. Readings were recorded at 570 nm using an ELISA plate reader (Biotek Elx800). Amphotericin B was taken as positive control. The experiment was performed in triplicate and the Leishmanial promastigote count was done using Neubauer chamber [26].

Antioxidant activity

Antioxidant activity was evaluated with the help of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following a reported method [27]. The crude extract (20 μl) and DPPH (180 μl) were mixed in a 96-well plate and incubated in dark for 60 minutes. The absorbance was recorded at 517 nm. Percent inhibition of the radicals was calculated from the equation given below:

$$Percent\ inhibition = \frac{Control\ OD - Sample\ OD}{Control\ OD}\ x\ 100$$

Ascorbic acid was used as negative control in different concentrations (μg/ml), while DMSO was used as the positive control.

Anti-cancer activity

Anti-cancer activity was assessed against malignant glioma U-87 (MG U87) cell lines [28]. MG U87 cells were rejuvenated from cryopreserved vials and cultured at 37 °C, in a supply of 5% CO_2. Streptomycin (100 μg/ml), penicillin (100 U/ml), and 10% Fetal bovine serum were added to a 1:1 mixture of Ham's F12 nutrient mixture media and Dulbecco's Modified Eagle Medium (DMEM:F12), in T25 flasks. The cells were transferred to flasks with larger capacity to enhance the cell number at a confluency of >80%. Growth inhibition assay was performed as reported earlier [29]. The MG U87 cells were transferred to 96-well plates (5000 cells/well). Hemocytometer was used for cell count. The cells were cultured in DMEM: F12, in a CO_2 incubator, for 24 hours with a supply of 5% CO_2. Different concentrations of the test sample were prepared in DMSO. The test sample was treated with the cell lines at different serial concentrations (0 (untreated control), 6.25, 12.5, 25, 50, 100, 200, and 400 μg/ml). Each sample was analyzed and all plates' patterns were repeated thrice. The cells were fixed in formalin (4%) after specified time of sample treatments. Crystal violet (0.1%) was used to stain the cells [30, 31], followed by washing with phosphate buffered saline and drying in air. Acetic acid (200 μl) was added to each well and absorbance was measured at 630 nm on a plate reader.

Results and Discussion

Phytochemical profiling of *M. coromandelianum*

The results of phytochemical screening indicated that methanolic extract contains proteins, terpenoids, carbohydrates, alkaloids, steroids, flavonoids, and reducing sugars (Table-1). Proteins are essential supplements to repair and maintain body, while terpenoids have been reported to be valuable in prevention and treatment of various diseases, such as cancer, microbial and viral infections, inflammation, spasmodic, parasitic, allergenic, and hyperglycemic diseases [32-34]. Carbohydrates and glycosides have been in use as dietary supplements to boost the immune system [35]. Steroids are beneficial for cholesterol-reducing properties and help in regulating the immune system of the body [36, 37]. Moreover,

alkaloids are well-known as anti-inflammatory and cardioprotective agents and also affect the central nervous system [38-40]. Thus, the pharmacological potential of the roots of this plant may be credited to these bioactive metabolites.

Table-1: Phytochemical screening of the roots of *M. coromandelianum.*

PHYTOCHEMICALS	Present(+)/Absent(-)
Proteins	+
Saponins	–
Terpenoids	+
Carbohydrate	+
Alkaloids	+
Steroid	+
Flavonoids	+
Glycosides	–
reducing sugars	+
phlobataninns	–

GC-MS analysis

The GC-MS analysis of *M. coromandelianum* roots revealed the presence of sixteen secondary metabolites, identified on the basis of high SI/RSI value (above 700) (Table-2). Nonanoic acid (2.31%), 6-methyl-octadecane (4.39%), hexadecanoic acid, methyl ester (2.79%), oleic acid (7.37%), 9,12-ctadecadienoic acid (7.37%), 1,2-benzenedicarboxylic acid diisooctyl ester (50.60%), [1,1':4',1''-terphenyl]-2,2''-diamine (4.83%), eicosane (0.73%), sitosterol (0.45%), Ar-turmerone (0.27%), 7-methyl-*Z*-tetradecen-1-ol acetate (1.53%), neopentyl glycol dibenzoate (0.38%), *Z*-(13,14-epoxy)tetradec-11-en-1-ol acetate (0.50%), 1-monolinoleoylglycerol trimethylsilyl ether (0.14%), and 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol (0.48%) were identified through GC-MS analysis. These isolates have been previously reported to possess antioxidant, anti-microbial, anti-cancer, cytotoxic, clonogenic, and anti-inflammatory activities (Table-2).

Biological activities

Anti-fungal activity

The methanolic crude extract of *M. coromandelianum* was tested against various fungal strains, such as *Aspergillus niger*, *Fusarium solani*, and *Candida albicans*, at 30, 50, and 100 μg/ml concentrations. The extract was found to be moderately active against *A. niger* and *F. solani*, exhibiting 16 mm (30 μg/ml), 18 mm (50 μg/ml), and 20 mm (100 μg/ml) zones of inhibition at corresponding concentrations against *A. niger* and 15 mm (30 μg/ml), 20 mm (50 μg/ml), and 25 mm (100 μg/ml) against *F. solani* (Table-3). This moderate anti-fungal potential may be attributed to the antagonistic effect of various chemical constituents [41-44]. Nevertheless, the results suggest that the roots might prove as an excellent source of some potent anti-fungal molecules.

Table-2: GC-MS analysis of roots of *M. coromandelianum.*

S.NO.	SI/RSI	Retention time	Compound	Peak area %	Bioactivity	References
1	**537/818**	**9.36**	**Nonanoic acid**	**2.31**	**Anti-microbial**	**[53, 54]**
2	**674/790**	**18.95**	**6-methyl-Octadecane**	**4.39**	**Antioxidant**	**[55]**
3	**834/859**	**18.34**	**Hexadecanoic acid, methyl ester**	**2.79**	**Antioxidant, nematicide**	**[56]**
4	**672/701**	**18.67**	**α-(acetyloxy)-2-hexyl-cyclopropanedecanoic acid methyl ester**	**0.16**	**Anti-microbial**	**[57]**
5	**661/761**	**20.27**	**Oleic Acid**	**7.37**	**Anti-cancer**	**[58]**
6	**679/754**	**20.27**	**(Z,Z)-9,12-Octadecadienoic acid**	**7.37**	**Anti-cancer, Anti-microbial, Antioxidant, anti-inflammatory**	**[59, 60]**
7	**909/912**	**22.39**	**1,2-Benzenedicarboxylic acid, diisooctyl ester**	**50.60**	**Anti-bacterial, cytotoxic, clonogenic**	**[60,61]**
8	**758/787**	**23.09**	**[1,1':4',1''-Terphenyl]-2,2''-diamine**	**4.83**	**-**	**–**
9	**71/897**	**17.00**	**Eicosane**	**0.73**	**Anti-microbial**	**[62]**
10	**852/879**	**26.00**	**β-sitosterol**	**0.45**	**Anti-inflammatory, Analgesic, Antioxidant**	**[63-65]**
11	**500/750**	**14.77**	**Ar-turmerone**	**0.27**	**Anti-fungal**	**[66]**
12	**733/788**	**20.99**	**7-Methyl-Z-tetradecen-1-ol acetate**	**1.53**	**Anti-fungal**	**[67]**
13	**670/911**	**12.41**	**Neopentyl glycol, dibenzoate**	**0.38**	**-**	**-**
14	**728/771**	**17.48**	**Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate**	**0.50**	**Antioxidant, Anti-cancer, Anti-microbial, anti–inflammatory, nematicide, Anti-histaminic, Anti-eczemic**	**[68]**
15	**662/740**	**27.32**	**1-Monolinoleoylglycerol trimethylsilyl ether**	**0.14**	**Cytotoxic, Anti-cancer**	**[68]**
16	**637/762**	**24.00**	**(3*α*,5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol**	**0.48**	**Anti-microbial**	**[68]**

Table-3: Anti-fungal activity of the crude extract of *M. coromandelianum* roots.

Fungal Strain	Concentration of Sample (μg/ml) vs. Zone of Inhibition (mm)			Positive Control
	30 μg/ml	50 μg/ml	100 μg/ml	Fluconazole
Aspergillus niger	10 mm	18 mm	20 mm	18.5
Fusarium solani	15 mm	20 mm	25 mm	16.4
Candida albicans	0 mm	0 mm	0 mm	18.2

Table-4: Anti-bacterial activity of the roots of *M. coromandelianum.*

Bacterial Strain	Concentration of Sample (μg/ml) vs. Zone of Inhibition (mm)			Positive Control
	30 μg/ml	50 μg/ml	100 μg/ml	Ofloxacin (5 μg/ml)
Klebsiella pneumonia	10 mm	12 mm	15 mm	30 mm
Escherichia coli	7 mm	18 mm	20 mm	26 mm
Staphylococcus aureus	12 mm	15 mm	25 mm	21 mm
Proteus mirabilis	10 mm	13 mm	15 mm	32 mm
Bacillus subtilis	11 mm	13 mm	17 mm	31 mm

Anti-bacterial activity

The anti-bacterial potential for the roots of *M. coromandelianum* was demonstrated at different concentrations (30, 50, and 100 μg /ml), against five selected multi-drug resistant bacterial strains, i.e., *Bacillus subtilis* (ATCC 6051), *Klebsiella pneumoniae* (ATCC 13883), *Proteus mirabilis*, *Staphylococcus aureus* (ATCC 12600), and *Escherichia. coli* (ATCC 8739). The crude extract exhibited maximum potential against *S. aureus* with zones of inhibition of 12, 15, and 25 mm at 30, 50, and 100 μg/ml, respectively, as compared to standard ofloxacin (21 mm at 5 μg/ml) (Table-4), while rest of the strains; *B. subtilis* (11, 13, and 17 mm vs control; 32 mm), *K. pneumoniae* (10, 12, and 15 mm vs control; 30 mm), *P. mirabilis* (10, 13, and 15 mm vs control;32 mm), and *E. coli* (7, 18, and 20 mm vs control; 26 mm) showed moderate activity. The activity may be attributed to the presence of sterols, sterol glycosides, and alkaloids [43, 45-47].

Anti-leishmanial activity

Leishmaniasis is a neglected parasitic disease. Its cases are on a rise throughout the world. Researchers are trying to reduce the resistance in the endemic areas, using alternative strategies, including herbal medicine. The results of the present study showed that the methanolic extract of *M. coromandelianum* roots has moderate activity against the *L. tropica* promastigotes at 250 (47%), 500 (50%), and 1000 μg/ml (67 %) concentrations, owing to the presence of chemical constituents such as, terpenoids, alkaloids, steroids, and flavonoids. Dihydrobetulinic acid (terpene) has been found to exhibit significant activity against visceral leishmaniasis as it induces apoptosis in *L. donovani* through its interaction with DNA topoisomerase I and II and inhibition of the DNA cleavage [48]. Flavonoids have been reported to cause apoptosis in *Leishmania* parasites through morphological changes in cells [49]. Several studies have demonstrated the Leishmanicidal potential of quercetin, fisetin, 3-hydroxy flavone, and luteolin [50]. The use of alkaloids against promastigotes dates back to the 1980s, when berberine chloride was found active against leishmaniasis. This compound reportedly inhibits the respiration process in growing amastigotes [46]. Results of the present study validate the leishmanicidal potential of the methanolic extract of the roots of *M. coromandelianum*, which indicates that the secondary metabolites in the extract possess significant Leishmanicidal properties. Thus, the roots of the plant can prove to be one of the best sources of natural and safe agents for the treatment of leishmaniasis [51].

Antioxidant activity

In preliminary exploration, the extract proved to be a significant *in vitro* DPPH radical scavenger (78 %) in a dose-dependent trend, in comparison to the standard Ascorbic acid (88 %). Extensive research has shown that medicinal plants have active phytochemical substances such as flavonoids which may contribute to the antioxidant potency. Furthermore, our findings suggest the use of *M. coromandelianum* as a constituent in herbal medicine for alleviating health conditions caused by oxidative stress [52].

Anti-cancer activity

Anti-cancer potential of the crude extract was evaluated against MG U87, malignant gliomas cell lines. The extract was found to be weakly active against the selected cancer cells. The control with no extract showed 100% survival rate. The survival rate decreased gradually with increasing concentration. Minimum average survival of the cells was achieved at 400 μg/ml (82.28 %). Oleic acid (HT-29), 9,12-octadecadienoic acid, and other compounds, detected in the extract through GC-MS analysis, have been reported for their anti-cancer activity earlier. The results suggest that the roots of *M. coromandelianum* can be a good source of molecules with anti-cancer capabilities.

Hemolytic activity

In vitro hemolytic activity of the roots extract of *M. coromandelianum* was performed at 250, 500, and

1000 μg/ml concentrations, revealing that the roots of *M. coromandelianum* are non-toxic even at high concentrations, such as 1000 μg/ml (28.27%), indicating that the root extract is very much safe, even at higher concentrations. Furthermore, the hemolytic activity of root extract is related to its chemical compositions. Thus, it has no effect on the stability of the erythrocyte membrane, making it non-toxic and suitable for the development of herbal medicines that are involved in the treatment of human ailments.

Conclusion

The current study was aimed at exploring the chemical profile and biological potential of the roots of *M. coromandelianum.* Preliminary studies revealed the presence of a variety of phytochemical classes. The presence of specific phytoconstituents was determined with the help of by GC-MS analysis. Moreover, the crude extract also exhibited good to moderate inhibitory potential against selected fungal and bacterial strains. In addition, the crude extract also exhibited anti-leishmanial properties against the *L. tropica* promastigotes at different concentrations. The extract was also found to be an excellent source of radical scavengers, as indicated by the results of DPPH radical scavenging assay. Furthermore, the extract was found to be non-toxic to human blood erythrocytes even at higher concentrations. The plant thus holds potential for the discovery of non-toxic phytomedicine. More in-depth studies on the plant are highly recommended for the isolation of pharmacologically important molecules which may prove as lead molecules.

Declaration of Conflict of Interest

The authors declare that there are no competing interests associated with this work.

References

1. S. Visht and S. Chaturvedi, Isolation of natural products, *J. Curr. Pharma Res.*, **2**, 584 (2012).
2. S. K. Singh, V. K. Singh, D. Kumar, A. Kumar, *Endophytes of Medicinal Plants: Diversity and Bioactivity*, in *Bacterial Endophytes for Sustainable Agriculture and Environmental Management*. 2022, Springer. p. 117.
3. S. Panda and A. Kar, Role of a gitogenin-type steroidal saponin (3-O-β-d-glucopyranosyl (1→ 2)-β-d-glucopyranosyl (1→ 4)-β-d-galactopyranoside-25R, 5α-spirostane-2α, 3β-diol), isolated from the leaves of *Malvastrum coromandelianum* in regulating thyrotoxicosis in rats, *Bioorg. Med. Chem. Lett.*, **26**, 4804 (2016).
4. C. Sittiwet, S. Jesadanont, P. Pongpech, P. Naenna, and P. Pongsamart, Antibacterial activity of *Malvastrum coromandelianum* Garcke against methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus, Curr. Res. Bacteriol.*, **1**, 42 (2008).
5. M. Islam, E. A. Muhammad, M. A. Saeed, M. Jamshaid, and M. T. J. Khan, Antimicrobial and irritant activities of the extracts of *Malva parviflora* L., *Malvastrum coromandelianum* L. and Amaranthus viridis L.–a preliminary investigation. *Pak. J. Pharm*, *20*(1&2), pp.3-6,(2010).
6. A. Yadav and S. Mohite, Anticancer activity and In-Silico ADMET analysis of *Malvastrum coromandelianum, Int J Pharm Sci Res.*, **11**, 71 (2020).
7. A. Yadav, P. Honmane, M. Bhosale, A. Chitruk, P. Rode, R. Birajdar, G. Vambhurkar, Antifungal Activity o*f Malvastrum Coromandelianum* Leaf Extracts, *Int. J. Pharm. Sci. Res.*, **5**, 01 (2020).
8. A. R. Yadav and S. K. Mohite, Antioxidant activity of *Malvastrum coromandelianum* leaf extracts, *Research J. Topical and Cosmetic Sci*, **11**, 59 (2020).
9. A. Yadav, S. Patil, V. Dharanguttikar, and S. Mohite, Anthelmintic Activity of *Malvastrum Coromandelianum* Leaf Extracts against *Pheretima Posthuma* and *Ascardia Galli, Int J Sci Res Chemi*, **5**, 18 (2020).
10. S. Saxena and P. Rao, GC-MS screening of bioactive constituents and antioxidant profiling in an invasive weed, *Malvastrum coromandelianum* (L.) Garcke, *Pharma Innovation*, **7**, 738 (2018).
11. S. Rattanajarasroj, J. Bansiddhi, A. Kun-Anake, B. Chaorai, Hypoglycemic activity of daikhad *Malvastrum coromandelianum* (L.) Garcke) in rats, *Warasan Krom Witthayasat Kan Phaet*, (2005).
12. Y. Liu, X. Su, Y. Xiang, X. Wang, P. Lai, Chemical composition, antibacterial, cytotoxic and antioxidant activities of the essential oil of *Malvastrum coromandelianum* aerial parts, *J. Essent. Oil-Bear. Plants*, **22**, 1040 (2019).
13. V. A. Soundatti, F. A. Tamboli, H. N. More, K. N. Tarkasband, Physicochemical Screening and antibacterial activity of *Malvastrum coromandelianum* (L.) Garcke, *European J. Biomed. Pharm. Sci.*, **8**, 406 (2021).
14. D. L. Latorre and F. A. Latorre, Plants used by the Mexican Kickapoo indians., *Econ. Bot.*, **31**, 340 (1977).
15. M. K. Sebastian and M. Bhandari, Medico-ethno botany of mount Abu, Rajasthan, India., *J. Ethnopharmacol.*, **12**, 223 (1984).
16. S. Devi, A. K. Lakhera, and V. Kumar, Structural analysis and antioxidant activity of an arabinoxylan from *Malvastrum coromandelianum* L.(Garcke), *RSC advances*, **9**, 24267 (2019).

17. M. Aderogba, B. Madikizela, and L. J. McGaw, Bioactive constituents from *Malvastrum coromandelianum* (L.) Garcke leaf extracts, *S. Afr. J. Bot.* , **126**, 371 (2019).
18. R. Yadav and M. Agarwala, Phytochemical analysis of some medicinal plants, *J. Phytol*, **3**, (2011).
19. S. Shah, S. M. M. Shah, Z. Ahmed, M. Yaseen, R. Shah, Phytochemicals, *in vitro* antioxidant, total phenolic contents and phytotoxic activity of *Cornu macrophylla* Wall bark collected from the Norh-West of Pakistan, *Pak. J. Pharm. Sci.*, **28**, 23 (2015).
20. A. Imtiaj and T.-S. Lee, Screening of Antibacterial and Antifungal activities from Korean wild Mushrooms., *World J. Agric. Sci.*, **3**, 316 (2007).
21. C. Valgas, S. M. D. Souza, E. F. Smânia, jr. A. Smânia, Screening methods to determine antibacterial activity of natural products, *Braz. J. Microbiol*, **38**, 369 (2007).
22. C. Anesini and C. Perez, Screening of plants used in Argentine folk medicine for antimicrobial activity, *J. Ethnopharmacol.*, **39**, 119 (1993).
23. S. Mehwish, H. Khan, A. U. Rehman, A. U. Khan, M. A. Khan, O. Hayat, N. Ullah, Natural compounds from plants controlling leishmanial growth via DNA damage and inhibiting trypanothione reductase and trypanothione synthetase: an in vitro and in silico approach, *3 Biotech*, **9**, 1 (2019).
24. B. Ahmad, A. Islam, A. Khan, M. A. Khan, I. Ul Haq, L. Jafri, N. Ullah, Comprehensive investigations on anti-leishmanial potentials of *Euphorbia wallichii* root extract and its effects on membrane permeability and apoptosis, *Comp. Immunol. Microbiol. Infect. Dis*, **64**, 138 (2019).
25. T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, . *Immunol. Methods.*, **65**, 55 (1983).
26. K. Adinehbeigi, M. H. Razi Jalali, A. Shahriari, and S. Bahrami,*in vitro* Antileishmanial activity of fisetin flavonoid via inhibition of glutathione biosynthesis and arginase activity in Leishmania infantum., *Pathog. Glob. Health.*, **111**, 176 (2017).
27. M. Zia, S. Gul, J. Akhtar, I. U. Haq, B. H. Abbasi, A. Hussain, M.F.Chaudhary, Green synthesis of silver nanoparticles from grape and tomato juices and evaluation of biological activities, *IET Nanobiotechnol.*, **11**, 193 (2017).
28. F. Sánchez-Férez, et al., Reactivity of homoleptic and heteroleptic core paddle wheel Cu (II) compounds, *Inorganica. Chim. Acta*, **487**, 295 (2019).
29. F. Sánchez-Férez, J. Soldevila-Sanmartín, J. A. Ayllón, T. Calvet, M. Font-Bardia, J. Pons, Synthesis and characterization of three new Cu (II) paddle-wheel compounds with 1, 3-benzodioxole-5-carboxylic acid, *Polyhedron*, **164**, 64 (2019).
30. M. Sanchez-Sala, J Pons, A. Alvarez-Larena, A., L.Bayés-García, M. Font-Bardia, J. A. Ayllón, Cu (II) 4-phenoxybenzoate dimers and monomer coordinated by pyridines: synthesis and crystal structures, *Polyhedron*, **151**, 545 (2018).
31. R. Sarma and J. B. Baruah, A mixed carboxylate polymer as an intermediate during synthesis of mononuclear bis-3, 5-dimethylpyrazole copper (II) benzoates, *J. Coord. Chem* . **61**, 3329 (2008).
32. T. Ojala, S. Remes, P. Haansuu, H. Vuorela, R. Hiltunen, K. Haahtela, P. Vuorela, Antimicrobial activity of some coumarin containing herbal plants growing in Finland, *J. Ethnopharmacol.*, **73**, 299 (2000).
33. T. Rabi and A. Bishayee, Terpenoids and breast cancer chemoprevention, *Breast Cancer Res. Treat.*, **115**, 223 (2009).
34. K.-H. Wagner and I. Elmadfa, Biological relevance of terpenoids, *Ann. Nutr. Metab*, **47**, 95 (2003).
35. N. Theis and M. Lerdau, The evolution of function in plant secondary metabolites, *Int. J. Plant Sci.*, **164**, S93 (2003).
36. K. Aidoo-Gyamfi, et al., Estrone sulfatase and its inhibitors, *Anti-Cancer Agents Med. Chem.*, **9**, 599 (2009).
37. B. A. Shah, G. N. Qazi, and S. C. Taneja, Boswellic acids: a group of medicinally important compounds, *Nat. Prod. Rep.*, **26**, 72 (2009).
38. A. Saiyed, N. Jahan, S. F. Majeedi, M. Roqaiya, Medicinal properties, phytochemistry and pharmacology of *Withania somnifera*: an important drug of Unani Medicine., *J. Sci. Innov.*, **5**, 156 (2016).
39. J. Kurek, *Alkaloids: Their importance in Nature and Human life*. 2019: BoD–Books on Demand.
40. P. H. Gleick, Roadmap for sustainable water resources in southwestern North America, *Proc. Natl. Acad. Sci.*, **107**, 21300 (2010).
41. T. Zhao, P. Krokene, N. Björklund, B. Långström, H. Solheim, E. Christiansen, A.K. Borg-Karlson, The influence of Ceratocystis polonica inoculation and methyl jasmonate application on terpene chemistry of Norway spruce, Picea abies, *Phytochemistry*, **71**, 1332 (2010).
42. A. Schmidt, G. Zeneli, A. M. Hietala, G. C. Fossdal, P. Krokene, E. Christiansen, J. Gershenzon, *Induced chemical defences in conifers: biochemical and molecular approaches to studying their function*, in *Chemical ecology and phytochemistry in forest ecosystems*. 2005, Elsevier. p. 1.
43. C. D. Hufford, S. Liu, and A. M. Clark, Antifungal activity of *Trillium grandiflorum* constituents, *J. Nat. Prod.*, **51**, 94 (1988).
44. J. Cho, H. Choi, J. Lee, M. S. Kim, H. Y. Sohn, D. G. Lee, The antifungal activity and membrane-disruptive action of dioscin extracted from

Dioscorea nipponica, *Biochim. Biophys. Acta - Biomembr.*, **1828**, 1153 (2013).
45. I. M. Villaseñor, J. Angelada, A. P. Canlas, D. Echegoyen, Bioactivity studies on β-sitosterol and its glucoside, *Phytother. Res.*, **16**, 417 (2002).
46. A. K. Ghosh, F. K. Bhattacharyya, and D. K. Ghosh, *Leishmania donovani*: Amastigote inhibition and mode of actior of berberine, *Exp. Parasitol.*, **60**, 404 (1985).
47. H. Guinaudeau, M. Lebœuf, and A. Cavé, Aporphinoid alkaloids, *J. Nat. Prod.*, **57**, 1033 (1994).
48. S. Alakurtti, T. Heiska, A. Kiriazis, N. Sacerdoti-Sierra, C. L. Jaffe, J. Yli-Kauhaluoma, Synthesis and anti-leishmanial activity of heterocyclic betulin derivatives, *Bioorg. Med. Chem. Lett.*, **18**, 1573 (2010).
49. L. C. Manjolin, M. B. G. dos Reis, C. do Carmo Maquiaveli, O. A. Santos-Filho, E. R. da Silva, Dietary flavonoids fisetin, luteolin and their derived compounds inhibit arginase, a central enzyme in Leishmania (Leishmania) amazonensis infection, *Food Chem.*, **141**, 2253 (2013).
50. D. C. O. Gomes, M. F. Muzitano, S. S. Costa, B. Rossi-Bergmann, Effectiveness of the immunomodulatory extract of *Kalanchoe pinnata* against murine visceral leishmaniasis, *Parasitology*, **137**, 613 (2010).
51. I. A. C. Araújo, R. C. De Paula, C. L. Alves, K. F. Faria, M. M. de Oliveira, G. G. M. Takarada, S. M. da Silva, *In vitro* efficacy of isoflavonoids and terpenes against *Leishmania* (Leishmania) *infantum* and *L. amazonensis*, *Exp. Parasitol.*, 108383 (2022).
52. S. Mehwish, A. Islam, I. Ullah, A. Wakeel, M. Qasim, M. A. Khan, N. Ullah, *In vitro* antileishmanial and antioxidant potential, cytotoxicity evaluation and phytochemical analysis of extracts from selected medicinally important plants, *Biocatal. Agric. Biotechnol.*, 19, 101117 (2019).
53. N. Sahin, I. Kula, and Y. Erdogan, Investigation of antimicrobial activities of nonanoic acid derivatives, *Fresenius Environ. Bull.*, **15**, 141 (2006).
54. Y. W.Jang, J.Y.Jung, I.K. Lee, S.Y. Kang, B.S.Yun,Nonanoic acid, an antifungal compound from *Hibiscus syriacus Ggoma*, *Mycobiology*, **40**, 145 (2012).
55. H. Temiz and Z. Tarakçı, Composition of volatile aromatic compounds and minerals of tarhana enriched with cherry laurel (*Laurocerasus officinalis*), *J. Food Sci. Technol.*, **54**, 735 (2017).
56. D. J. Dr, Duke's Phytochemical and Ethnobotanical Databases, *Artemisia annua*, (2005).
57. J. Amiri Moghaddam,A. Dávila-Céspedes,S. Kehraus,M. Crüsemann, M. Köse, C.E Müller, G.M. König, Cyclopropane-containing fatty acids from the marine bacterium *Labrenzia* sp. 011 with antimicrobial and GPR84 activity, *Marine drugs*, **16**, 369 (2018).
58. O .D.DAILEY, X. Wang, F. Chen, G.Huang, Anticancer activity of branched-chain derivatives of oleic acid, *Anticancer Res.*, **31**, 3165 (2011).
59. L .S. Wei, W. Wee, J.Y.F. Siong, D.F. Syamsumir, Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract, *Acta Medica Iranica*, 670 (2011).
60. N. Yamabe,K.S. Kang,A.Y. Lee, D. Lee, J.M Choi, S. Lee, S. Lee, Identification of anti-cancer active components of *Taraxacum coreanum* on human gastric cancer AGS cells, *J. Korean Soc. Appl. Biol. Chem.*, **57**, 187 (2014).
61. L. A. Fadipe, A. Haruna, and I. Mohammed, Antibacterial activity of 1, 2-benzenedicarboxylic acid, dioctyl ester isolated from the ethyl acetate soluble sub-portion of the unripe fruits of *Nauclea latifolia*, (2014).
62. A. H. Al-Marzoqi, I. H. Hameed, and S. A. Idan, Analysis of bioactive chemical components of two medicinal plants *(Coriandrum sativum and Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS), *Afr. J. Biotechnol.*, **14**, 2812 (2015).
63. J. M. Prieto, M. C. Recio, and R. M. Giner, Anti-inflammatory activity of β-sitosterol in a model of oxazoloneinduced contact-delayed-type hypersensitivity, *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, **5**, 57 (2006).
64. E.J.Moon, M.Y.Lee,O.H, Lee, M.J. Lee, S.K. Lee, M.H. Chung, K.W. Kim, A ncovel angiogenic factor derived from Aloe vera gel: β-sitosterol, a plant sterol, *Angiogenesis*, **3**, 117 (1999).
65. M. Vivancos and J. J. Moreno, β-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages, *Free Radic. Biol. Med.*, **39**, 91 (2005).
66. A. Apisariyakul, N. Vanittanakom, and D. Buddhasukh, Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae), *J. Ethnopharmacol.*, **49**, 163 (1995).
67. N. Sana, A. Shoaib, and A. Javaid, Antifungal potential of leaf extracts of leguminous trees against *Sclerotium rolfsii*, *Afr. J. Tradit. Complement. Altern. Med.*, **13**, 54 (2016).
68. T. R. Elsayed, D. F. Galil, M. Z. Sedik, H. M. Hassan, M. W. Sadik, Antimicrobial and anticancer activities of actinomycetes isolated from Egyptian soils, *Int. J. Curr. Microbiol. Appl. Sci.*, **9**, 2020 (2020).