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Quantitative Estimation of Phytochemicaland Antimicrobial Properties of Crude Leaf Extract, MimusopselengiL.

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Abstract: ***The present investigation was done to estimate the secondary metabolites from the medicinally important plant, Mimusopselengi L. The quantitative estimation of bioactive molecules from the leaves showed the presences of flavanoids, terpenoids, saponins, flavonols and tannins. The results obtained clearly indicates that leaves contains highest amount alkaloids content is highest as 45.1±1.2 mg/g and the saponins contents is lowest as 0.589±0.07 mg/g. Acetone leaf extracts of Mimusopselengi show alkaloid is highest as 54.2±2.9 mg/g and the steroids is lowest as 2.0±0.31 mg/g. The chloroform leaf extracts of Mimusopselengi show saponins is highest as 33.1±2.8 mg/g and alkaloids is lowest as 0.486±0.01 mg/g. all values were positively significant at (P<0.01) 5% level. Secondary metabolite of crude leaf extracts of Mimusopselengi is range between 54.53±2.9 (alkaloids) to 0.486±0.01 mg/g. Hence leaves of Mimusopselengi served to have the potential source for pharmaceutical drug.***

Keywords: Mimusopselengi; Secondary metabolites; Flavonoids; Terpenoids; Tannins.

I. INTRODUCTION

Huge numbers of infectious diseases caused by the gram negative bacteria that are resistant to many commonly used antibiotics are the causes of great concern to the clinicians as well as the microbiologists. Phytomedicines, prepared from various plant materials, such as Ayurvedic traditional medicine, are comparatively safe, inexpensive and have less antagonistic effects. Leaf, bark, fruit and seeds of MimusopselengiL. possess several medicinal properties, viz., astringent and tonic in dental diseases and uterine disorders documented by [1., 2., 3. and 4.]. Plant has also been reported for analgesic, diuretic, antiulcer, antipyretic, anti-inflammatory and antimicrobial activities investigated by [5., 6., 7. and 8.].In rural areas of developing countries, like India, herbal materials are in use as the primary source of medicines [9.]. Nearly 80% of the people in developing countries use traditional drugs for the purpose of primary health maintenance [10.]. Among the plant species occurring worldwide [11.], only a very less percentage has been investigated phytochemically. Medicines of plant origin used by the medical practitioners are in the form of extract of the whole plants or part of the plants. Some of the effects elaborated by the plant extracts used in the traditional medicine include antiviral, antitumor, antimicrobial, and having central nervous system effect [12.]. Plants possess bioactive components of therapeutic value to cure several health disorders of humans [13.].

Research interest on the antimicrobial activity of plant extracts is a raising one because of the current problems with bacterial antibiotic resistance, and the use of phytochemicals as natural antimicrobials is gaining popularity [14]. One such important traditional medicinal plant is M. elengi belonging to the Sapotaceae family, called as ‘Bakula’ in Bengali and it is well known in Ayurvedic medicine. All the parts of M. elengi have medicinal properties, and the leaves are reported to be used in the treatment of bacterial diseases by tradition [15.]. Pharmacognostic and phytochemical screening reports on M. elengi stem bark has been documented [16.]. Recently, estimation of triterpene acids using from M. elengi stem bark has been published [17. and 18.]. Antimicrobial, antiviral and hepato protective and cytotoxic activities of M. elengi are well accepted because of the wealth of scientific literature supporting these effects [19.].

In view of these the present work undertaken to evaluate the in vitro antimicrobial activity, and to identify the phytochemical constituents the medicinally important plant, Mimusopselengi. However, the bioactive potential of compounds from Indian traditional medicinal plants has been little studied, especially in Marathwada region, Maharashtra state.

Therefore, In the present study report the quantitative estimation of phytochemicals and antimicrobial potential of the medicinally important plant, Mimusopselengi collected from Marathwada region, Maharashtra.

II. MATERIALS AND METHODS

A. Survey Of Sampling Stations

Survey of sampling stations was conducted different localities of Marathwada region, Maharashtra state (N $18^0$44'27.81" E $77^0$42'49.53"), India during the period of January 2015 to February 2017.

B. Collection Of Sample& Preparation Of Crude Extract

The leaf material of Mimusopselengi was collected by an eco-friendly. Samples were incised out and (Approx. 100 g) were washed with tap water, air dried and chopped into small size and extracted with 1000 ml (1:10) methanol, acetone, chloroform and hexane for about 7 days. Then extract was filtered through Whatmann paper No. 1 and solvent was removed by rotary vacuum evaporator (Buchi type-Superfit, Bangalore) under reduced pressure so as to get the crude sponge extract. The concentrated extract was used for further study.

C. Quantitative Estimation Of Phytochemicals

The plant crude extracts were screened for quantitative estimation of phytochemical properties of test medicinal plants using standard methods [20.]. At present, these species of medicinal plant is MimusopselengiL.(Leaf).

D. Quantitative Estimation Of Alkaloids

Determination of Alkaloids was done by standard method described by [21.]. The absorbance of the complex in chloroform was measured at 280 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

E. Quantitative Estimation Of Flavonoids

Total flavonoid content was determined by Aluminium chloride method [22.] using catechin as a standard. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

F. Quantitative Estimation Of Saponins

Determination of Saponins was done by standard method described by [21.]. Absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

G. Quantitative Estimation Of Steroids

1 ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20 C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

H. Quantitative Estimation Of Phenolic Compounds

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR) method reported by [23.]. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph.

I. Quantitative Estimation Of Tannins

The mixture was then made up to mark with water, mixed well and allowed to stand for 20min.A bluish-green colour was developed at the end of range 0-10ppm.The absorbance of tannin acid standard solution as well as sample shall be read after color development on a spectrophotometer at wave length of 760nm [24.].

J. Antibacterial Screening Of Test Plant Crude Extracts

The antibacterial assays were performed by agar well diffusion method is widely used to evaluate the antimicrobial activity of crude extracts [25., 26.]. *Escherichia coli*, *Salmonella* typhi, (Gram negative bacteria) *Bacillus* subtilis, *Staphylococcus* aureus, (Gram

positive bacteria) strains were used as test organisms (Table- 3). Icosonizid (10µg/ml) was used as positive control. The plates were incubated at 37°C for 24 hrs.

K. Antifungal Screening Of Test Plant Crude Extracts-

Assays were performed by agar well diffusion method. All plant crude extracts were tested against Aspergillusniger, Trichodermaharzianum, Alternariaburnsii and Fusariumoxysporum(Table- 3). Fluconazole (10µg/ml) was used as the positive control. The plant crude extract was loaded in to the well and to find out the inhibitory potential. The plates were incubated at 28°Cfor 48 hrs.

III. RESULTS

Phytochemicals of leaf crude extract of traditional medicinal plant, Mimusopselengishow the methanol and acetone crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils; as well as chloroform extract contains alkaloids, flavonoids, sterol and terpenoids, carbohydrates, fats and fixed oils. The phytochemical screening of leaf extracts of Mimusopselengirevealed that steroids, flavonoids, cathaholic, cardiac glycosides, phenolic compounds, carbohydrates and proteins were found in all crude extracts, while alkaloidswasabsent in chloroform crude extract (Figure- 2A; Table- 1).The table 1 shows the results of quantitative phytochemical screening, leaf extracts of Mimusopselengi show phytochemicals in the order of alkaloids>saponins> flavonoids> tannins> phenolic compounds> steroids (Figure- 1). The selection of type crude leaf extractsof Mimusopselengi for quantitative phytochemical determination was depends on the presence of highest concentration of alkaloids, terpenoids and saponins concentration (Table- 1). The results of preliminary qualitative analysis show highest concentration of same in chloroform, methanol and acetone leaf extracts of Mimusopselengi, shown in figure 2, 3 and 4 respectively.The quantitative determination of phytochemical show, methanolic leaf extracts of Mimusopselengi as given in table 1 and figure 3.D indicates that the alkaloids content is highest as 45.1±1.2 mg/g and the saponins contents is lowest as 0.589±0.07 mg/g. Acetone leaf extracts of Mimusopselengi12 and figure 23 show alkaloid is highest as 54.2±2.9 mg/g and the steroids is lowest as 2.0±0.31 mg/g. The chloroform leaf extractsof Mimusopselengi10 and figure 21 show saponins is highest as 33.1±2.8 mg/g and alkaloids is lowest as 0.486±0.01 mg/g. all values were positively significant at ($P<0.01$) 5% level. Secondary metabolite of crude leaf extracts of Mimusopselengi is range between 54.53±2.9 (alkaloids) to 0.486±0.01 mg/g.

The results of in vitro testing of crude leaf extract of MimusopselengiL. show antimicrobial activity against four human pathogenic bacteria as well as four plant pathogenic fungal species, table 2 and figure 5. Inhibition zones of crude extracts against the specific test organisms were measured in mm. The extract restricted the growth of pathogens on the media around wells. The 80% methanol crude leaf extract of MimusopselengiL.show maximum inhibition zone (1-4 mm) in the following manner Salmonella typhi>Trichodermaharzianum>Escherichia coli>Bacillus subtilis>Fusariumoxysporum>Aspergillusniger> Staphylococcus aureus>Alternariaburnsii; 80% acetone crude leaf extract of MimusopselengiL. show maximum inhibition zone (1-4 mm) in the following manner Trichodermaharzianum>Alternariaburnsii> Staphylococcus aureus>Aspergillusniger> Bacillus subtilis>Fusariumoxysporum>Escherichia coli>Salmonella typhi; (Table- 2 and Figure- 6, 7).80% chloroform crude leaf extract of MimusopselengiL.show maximum inhibition zone (1-3 mm) in the following manner Trichodermaharzianum>Escherichia coli>Fusariumoxysporum> Bacillus subtilis>Aspergillusniger>Alternariaburnsii>Staphylococcus aureus> Salmonella typhi; (Table- 2 and Figure- 6, 7).

IV. DISCUSSION

We could able to isolate two single compounds from the crude methanolic bark extract. These compounds were earlier reported from different parts of the M. elengi[3., 27.]. The Quercitol has been isolated from the aqueous fraction of the bark, where as we could able to isolate the same as insoluble fraction of the methanolic extract. Here the procedure for preparation of extracts is varying to the previously reported study. The compound 2 which is lupeol, has been previously reported in the ethanolic extract of heart wood of M. elengi. We have isolated the pure product in the methanolic extract of bark. In both the isolation procedures, the method of extraction and isolations differs. Thus, even though these compounds are previously reported from this plant, the procedure of isolation is different.In present study, phytochemicals of leaf crude extract of traditional medicinal plant, Mimusopselengi show the methanol and acetone crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils; as well as chloroform extract contains alkaloids, flavonoids, sterol and terpenoids, carbohydrates, fats and fixed oils. It revealed that steroids, flavonoids, cathaholic, cardiac glycosides, phenolic compounds, carbohydrates and proteins were found in all crude extracts, while alkaloids was absent in chloroform crude extract. And the

results of quantitative phytochemical screening, leaf extracts of Mimusopselengi shows in the order of alkaloids>saponins> flavonoids> tannins>phenolic compounds> steroids The triterpenoids are considered as potent biological molecules, due to their wide spectrum of biological activities. Thus all the biological activities were earlier reported for the isolated compounds we did not perform the biological screening of the isolated product.The present study show alkaloids content is highest as 45.1±1.2 mg/g and the saponins contents is lowest as 0.589±0.07 mg/g. Acetone leaf extractsof Mimusopselengi12 and figure 23 show alkaloid is highest as 54.2±2.9 mg/g and the steroids is lowest as 2.0±0.31 mg/g. The chloroform leaf extracts of Mimusopselengi10 and figure 21 show saponins is highest as 33.1±2.8 mg/g and alkaloids is lowest as 0.486±0.01 mg/g. all values were positively significant at ($P<0.01$) 5% level. Secondary metabolite of crude leaf extracts of Mimusopselengi is range between 54.53±2.9 (alkaloids) to 0.486±0.01 mg/g.The amount of bioactive molecules including flavonoids, phenolic compounds, alkaloids, and terpenoids was previously reported for their cancer properties were identified from this plant in several studies [28.]. Among those isolated bioactive molecules, Lupeol, betulinic acid, gallic acid, and taraxerol have been shown in several reports to possess anti-cancer activity [29., 30.]. In addition, many numbers of bioactive molecules were isolated and identified from the leaf and bark of this plant [3.]. Therefore, the anti- inflammatory and anti-cancer activity observed in crude leaf and bark extracts may be specifically due to the isolated bioactive molecule or it may due to other phytochemical constituents present. To conclude, according to the results obtained M. elengi leaf and bark extracts appeared to be potent anti-inflammatory and anti-cancer agent.

There are different ways in which a phytochemical can work. It can act as an antioxidant and protect cells against free radical damage, eg. polyphenols, carotenoids etc. It can stimulate certain enzymes there byreduce risk for breast cancer, eg. terpenes[31.].Preliminary phytochemical studies are helpful in finding out chemical constituents in the plantmaterial that may well lead to their quantitative estimation. Recently much attention has been directed towards extracts and biologically active compounds isolated from popular plant species. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances ofdifferent compositions which occur as secondary metabolites [32.].For the preliminary phytochemical analysis, leaf extracts (petroleum ether, chloroform and water) of MimusopselengiL. were taken.

The results of the present study coincides with that of previous study [33.], who conducted thephytochemical investigation of aqueous and methanolic extracts of two medicinal plants (SpathodeacampanulataP. and TridaxprocumbensL). The phytochemical screening revealed the presence of alkaloids, tannin, saponin, steroids, terpenoid and falvonoids. [34.] Study the phytochemicalanalysis on leaves of Euphorbia hirtaL. and the results showed the presence of alkaloids, steroids, carbohydrates and flavonoids. Phytochemical investigation of antidiabetic plant scopariadulcisL. (Scrophulariaceae) grown inNigeria, revealed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, steroids andterpenes[35.].

In the present study, results of in vitro testing of methanol crude leaf extract of MimusopselengiL. show antimicrobial in the following manner Salmonella typhi>Trichodermaharzianum>Escherichia coli>Bacillus subtilis>Fusariumoxysporum>Aspergillusniger> Staphylococcus aureus>Alternariaburnsii; 80% acetone crude leaf extract of MimusopselengiL. show maximum inhibition zone (1-4 mm) in the following manner Trichodermaharzianum>Alternariaburnsii> Staphylococcus aureus>Aspergillusniger> Bacillus subtilis>Fusariumoxysporum>Escherichia coli>Salmonella typhi. Our results were comparable to those obtained by Muanda et al. (2011) with methanol extract of V. doniana. The higher inhibition percentage of methanol and hydroethanol extracts could be due to the presence of tannins and flavonoids. It has been reported that flavonoids have the ability to inhibit spore germination of plant pathogens [36.], and tannins inhibit the germ-tube formation and stimulus of phagocytosis by macrophages [37.], and extracellular microbial enzymes through inhibition of oxidative phosphorylation [38.].

V. CONCLUSION

In conclusion, plant has numerous ways to combat the intrinsic, extrinsic and environmental factors. They have developed morphological, physiological and biochemical techniques to save themselves in this environment. Hence, the bioactive substances produced by them are in one way unique and essential for their survival. These bioactive substances are utilised by them in their various spheres of living like, growth, reproduction, protection, defense etc. which have complex structural composition and found to have functional significance to humans. The results showing the activity of leaf extract of MimusopselengiL. against some potential human and plant pathogens. Some of the substances identified in leaves of MimusopselengiL. are definitely produced within the plant in sufficient amounts to display their biological activities. The data from available literatures reveal that the marine ecosystem is not only the resources to discover various bioactive agents but also an avenue to identify new cellular targets for drug discovery. A proactive interaction among research scholars, scientists, pharmaceutical sector, and government regulating authorities is important to the incorporation of this challenging new bioactive agent in clinical applications.

Future studies on MimusopselengiL. may serve as a rich source of information as well as pharmaceuticals significance. In this context recent finding with MimusopselengiL. may usher a new field of processing bioactive substances from marine invertebrate available in Marathwada region.

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	Phytochemicals					
Extractants	**Alkaloids**	**Steroid**	**Flavonoid**	**Saponins**	**Phenolic compound**	**Tannin**
Chloroform 80%	0.48±0.01	4.32±0.1*	3.6±2.1	33.1±0.1**	7.86±0.47**	6.12±1.2**
Methanol 80%	45.1±2.6**	2.9±0.7	14.2±1.4**	0.589±0.81	3.94±0.56	8.9±1.89**
Acetone 80%	54.2±5.41**	2±0	22.7±0.94**	19.7±1.2**	11±1.5*	9.4±0.64**

Values are expressed in average of triplicate and mean ±Standard deviation
** = significant value at 1% ($p < 0.01$), * = significant value at 5% ($p < 0.05$)
Table -1:Preliminary phytochemical screening of leaf crude extracts of*Mimusopselengi.fruticosa.*

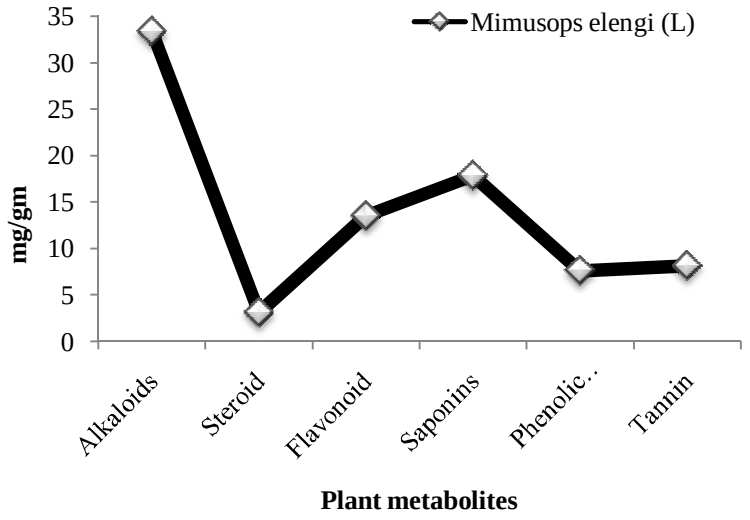


Figure- 1:Showing concentration of plant metabolites in mg/g from leaf crude extractof *Mimusopselengi*L.

Plant extract	Solvent extract	Antimicrobial activity diameter of zone of inhibition in mm							
		E. coli	*S. typhi*	*S. aureus*	*B. subtilis*	*A. burnsii*	*F. oxysporum*	*A. niger*	*T. harzianum*
Mimus opsele ngi L. (Leaf)	Aqueous	1.10±0.1	2.22±0.10	1.11±0.0	1.78±0.2	1.17±0.08	2.37±0.09	2.33±1.4	2.88±0.78
	Ethanol 80%	2.11±0.10	2.42±0.10	1.21±0.0	2.4±1.0	2.0±0.20*	3.12±0.40	1.5±1.12	2.33±0.63
	Chloroform 80%	3.17±0.08*	1.23±0.2	1.33±1.2	2.7±2.3	4.53±0.11*	8.3±0.13**	11.5±0.47**	14.3±0.77**
	Methanol 80%	4.0±0.20*	5.0±0.10**	8.1±1.10**	11.7±0.2**	5.50±0.15**	1.23±0.31	4.23±0.78*	5.61±1.66**
	Hexane 80%	3.13±0.11*	4.23±0.48*	2.13±0.18	2.1±0.5	1.34±1.3	3.12±0.40*	4.61±0.6*	6.32±1.7**
	Acetone 80%	2.13±0.5	5.1±1.5**	4.23±1.4*	6.13±1.6**	1.11±0.10	2.34±0.30	2.34±0.4	2.45±0.64

Values are expressed in average of triplicate and mean ± Standard deviation
** = significant value at 1% ($p < 0.01$), * = significant value at 5% ($p < 0.05$)
Table- 1: Showing zone of inhibition in mm of all crude extracts of all test plants against human and plant pathogens.

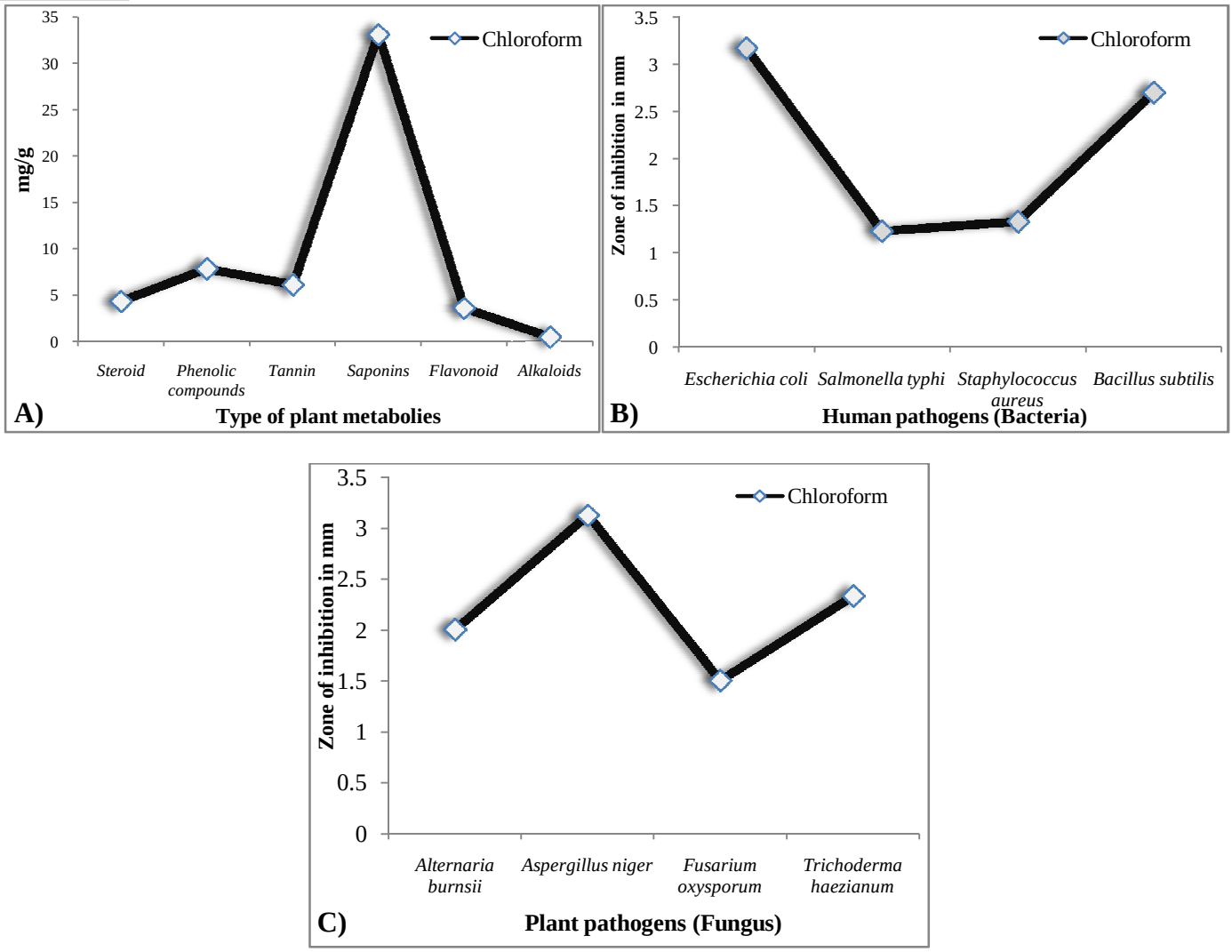
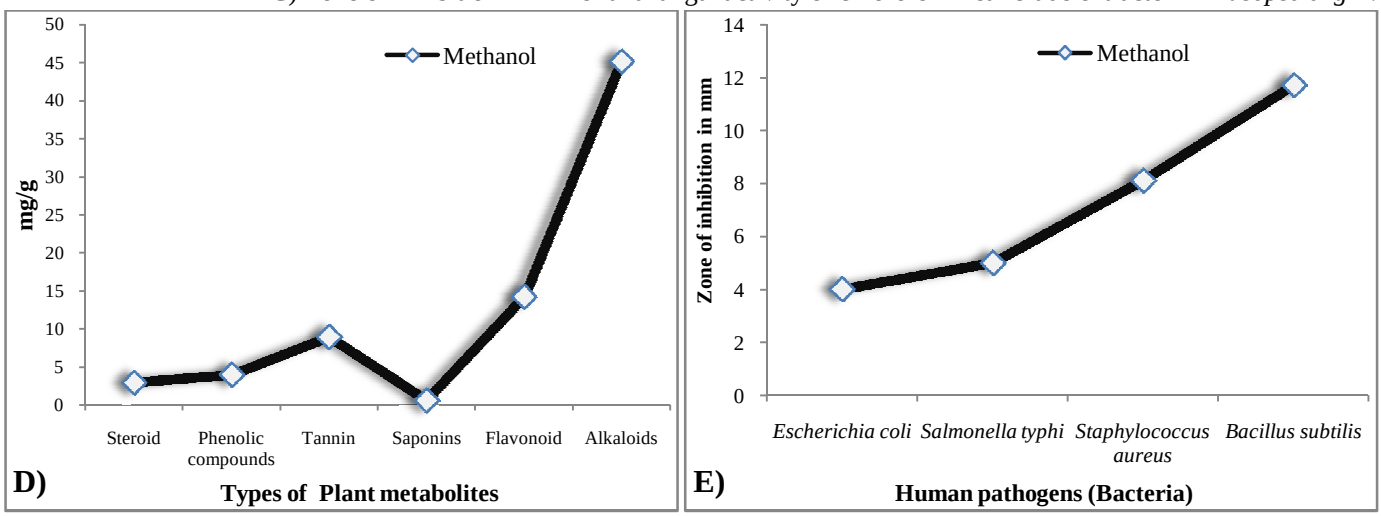


Figure- 2: Showing: A) Concentration of plant metabolites in mg/g from chloroform leaf crude extractof *Mimusopselengi*L.
B) Zone of inhibition in mm of antibacterial activity of chloroform leaf crude extractof *Mimusopselengi*L.
C) Zone of inhibition in mm of antifungal activity of chloroform leaf crude extractof *Mimusopselengi*L.



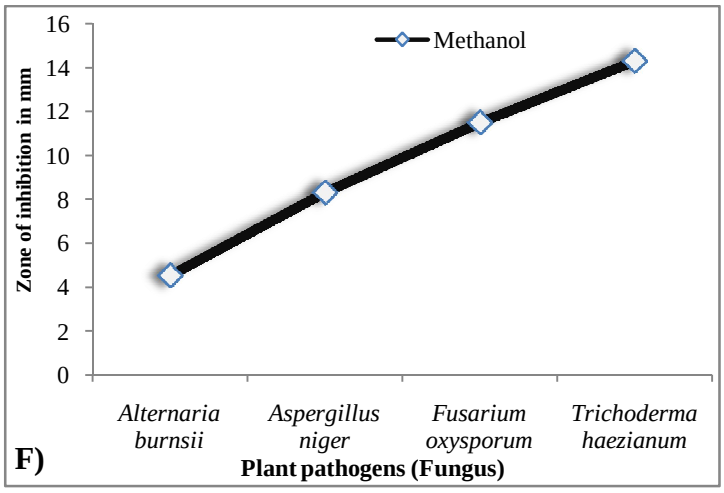
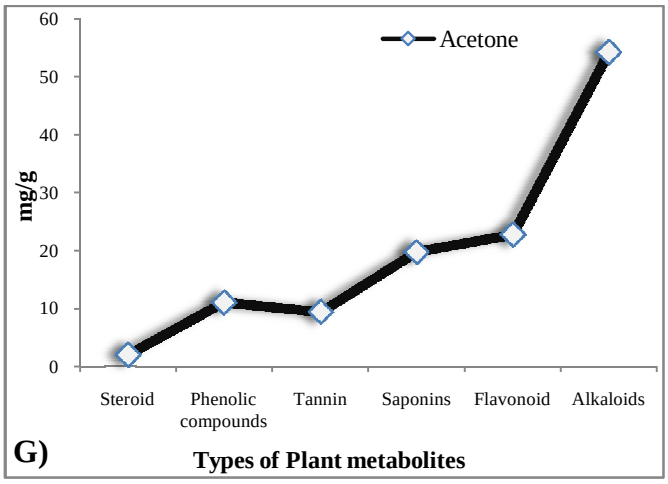
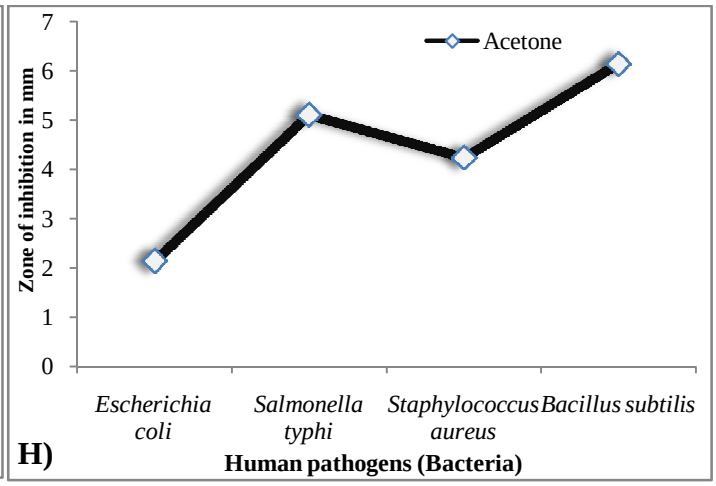


Figure- 3: Showing:D) Concentration of plant metabolites in mg/g from methanol leaf crude extractof *Mimusopselengi*L.
E) Zone of inhibition in mm of antibacterial activity of methanol leaf crude extractof *Mimusopselengi*L.
F) Zone of inhibition in mm of antifungal activity of methanol leaf crude extractof *Mimusopselengi*L.





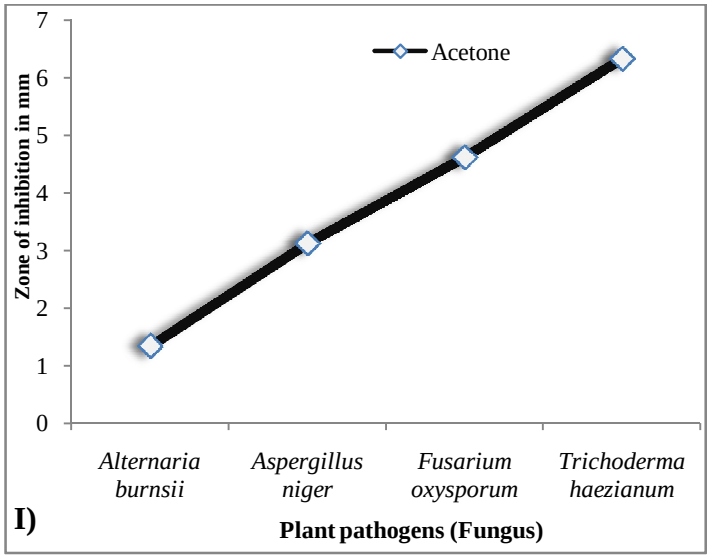


Figure- 4: Showing:G) Concentration of plant metabolites in mg/g from acetone leaf crude extractof MimusopselengiL.
H) Zone of inhibition in mm of antibacterial activity of acetone leaf crude extractof MimusopselengiL.
I) Zone of inhibition in mm of antifungal activity of acetone leaf crude extractof MimusopselengiL.

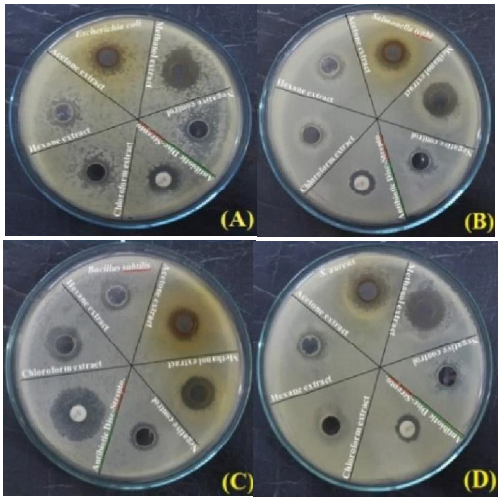


Figure- 5: Showing antibacterial assays of crude extract of Mimusopselengi L. against human pathogens [(A) Escherichia coli, (B) Salmonella typhi, (C) Bacillus subtilis and (D) Staphylococcus aureus].

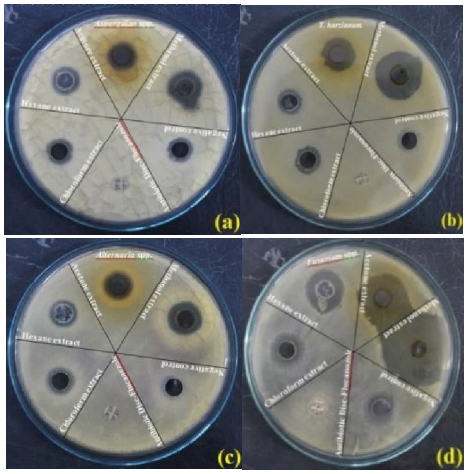
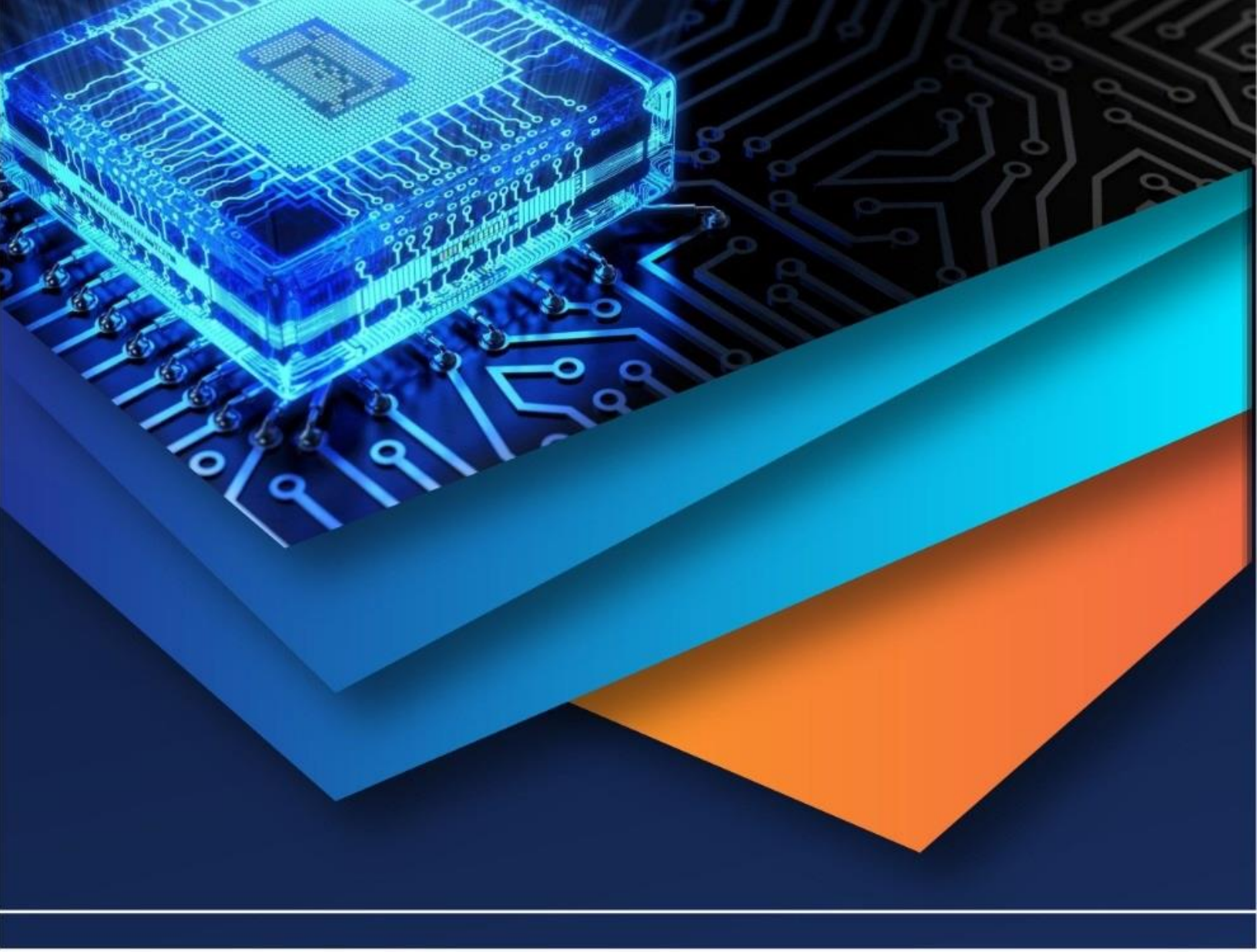


Figure- 6: Showing antifungal assays of all crude extracts of Mimusopselengi L. against plant pathogens [(a) Aspergillusniger, (b) Trichodermaharzianum, (c) Alternariaburnsii and (d) Fusariumoxysporum].



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