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ANTIMICROBIAL POTENTIAL OF CRUDE EXTRACTS AND ISOLATES OF ROOTS OF *MIMOSA PUDICA* LINN. COLLECTED FROM THE LOCALITY OF MOHUDA ENVIRON, GANJAM, ODISHA

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ABSTRACT

A complete documentation of the ethno-biological information presently existing among the diverse communities is needed at an earliest so as to restore the traditional medicinal knowledge. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified as being evidenced from many cited literatures. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status. Hence, the plant, *Mimosa pudica* Linn. was selected for the present study based on its medicinal use in the locality. Successive solvent extraction of the root powder of *M. pudica* was carried out as per the standard procedure using various solvents like, petroleum ether (40^{o}-60^{o}C), ethyl acetate and methanol. Preliminary phytochemical analysis, isolation of metabolites and antimicrobial activities of the extracts as well as the first time reported isolates were carried out. Preliminary phytochemical studies of vaious solvent extracts revealed presence of many biologically important secondary metabolites, such as, alkaloids, steroids, phenolic compounds, tannins, flavonoids, triterpenoids, carbohydrates, cardiac glycosides and amino acids. Twelve numbers of bacteria and fungi were used in the present study to identify the antimicrobial potency of the isolates as well as the root extracts of *M. pudica* in comparison to the control standards. The ethyl acetate root extract (EAE) exhibited better inhibition of bacteria and fungi in comparison to other two extract (petroleum ether and methanol). The isolated compound, MPR-03 showed better inhibitions against selective microbes and fungi.

INTRODUCTION: The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries is fast disappearing from the face of the earth due to the advent of lack of documentation, modern technology and transformation of traditional culture. A complete documentation of the ethno-biological information presently existing among the diverse communities is needed at an earliest so as to restore the traditional medicinal knowledge. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified as being evidenced from many cited literatures. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status.

According to an all India Ethno botanical Survey conducted by the Ministry of Environment (1985-90), there are nearly 6000 species of medicinal plants in India which are being used by the traditional practitioners in tribal areas and other village communities. Utilization of plants for medicinal purposes in India has been documented long back in ancient literatures [1]. However, organized studies in this direction were initiated in 1956 [2] and off late such studies are gaining momentum and popularity due to endangered status of traditional knowledge and the declining phyto-diversity.

Presently in the developing countries, synthetic drugs are not only of much cost effective and inadequate for the treatment of diseases; but are also often with adulterations and side effects [3]. It is therefore, essential to search for the efficacious plants of medicinal value for better manifestations. *M. pudica* is abundantly available in the locality of Mohuda environ (Ganjam), Odisha and after self-investigation and interaction with the local inhabitants of Mahuda, it was noted that the people are using the plant both externally and internally for treatment of various ailments like skin itching, cut wound healing, diarrhoea and dysentery. Hence, the plant was selected by the author to carry out the present research work on phytochemical examination and antimicrobial screening so as to establish its inherent medicinal properties which might ultimately help the nation in availing newer phyto-medicines for antimicrobial therapy.

Mimosa pudica Linn., a creeping annual (perennial herb under undisturbed ecology), originally a native of Brazil, got naturalized especially found abundant in hotter regions of India, commonly considered as a weed (grass). It occurs mostly in open marshy waste land areas [4,5]. *M. pudica* has also been found in crop-lands, orchards, pastures including roadsides and areas disturbed by construction. It may grow singly or entangled thickets [6].

MATERIALS AND METHOD:

Collection of Plant material: The fresh roots from young matured plants of *Mimosa pudica* Linn. were collected from the locality of Mohuda environ (Ganjam), Odisha. After authentication, the roots were washed under running tap water to remove adhering dirt followed by rinsing with distilled water and then shade dried and pulverized in a mechanical grinder followed by sieving (sieve no. 40) to obtain coarse powder.

Preparation of extracts: The dried powdered roots (500 g) were separately extracted successively with various solvents *viz.* petroleum ether (40^{o}-60^{o}C), ethyl acetate and methanol in increasing order of polarity using a Soxhlet extractor. The period of extraction was fixed at 48 h for every solvent at every stage of the extraction process. The solvents were purified by distillation prior to extraction [7, 8]. After completion of extraction, the color, consistency and extractive value of individual extract was determined with respect to the dried plant material (**Table 1**).

TABLE 1: RESULTS SHOWING COLOR, CONSISTENCY AND EXTRACTIVE VALUES OF DIFFERENT EXTRACTS OF *M. PUDICA* ROOT

Solvents used	Color of Extracts	Consistency	% w/w of Extracts*
Petroleum ether (40-60^{0} C)	Dark green	Greasy	0.72±0.03
Ethyl acetate	Light yellow	Greasy	1.11±0.21
Methanol	Brown	Sticky	3.38±0.28

*Results expressed as Mean ± SEM from three observations.

Qualitative phytochemical analysis of various extracts: Petroleum ether (40^{o}–60^{o}C), ethyl acetate and methanol extracts obtained from the above extraction processes were analyzed for various phyto-constituents present in these extracts by the method of qualitative phytochemical analysis [9, 10, 11] and the results were depicted in **table 2**.

TABLE 2: RESULTS OF PRELIMINARY PHYTOCHEMICAL TESTS OF VARIOUS EXTRACTS OF *M. PUDICA* ROOT

Test For	P.E. Ext.	E.A. Ext.	M. Ext.
Alkaloids	+	-	-
Carbohydrates	-	-	-
Cardiac glycosides	-	-	+
Phenolic compounds and Tannins	-	+	-
Proteins and Amino acids	-	-	+
Gums and Mucilage	-	-	+
Flavonoids	-	+	-
Saponins	-	-	+
Steroids	+	-	-
Triterpenoids	-	+	-

P.E. Ext. – Petroleum ether extract, E.A. Ext. – Ethyl acetate extract, M. Ext. – Methanol extract. '+': Present & '-': Absent

Phytochemical examination of selected extract

The ethyl acetate root extract of *M. pudica* was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with the various solvent ratios of n-hexane: ethyl acetate and then with ethyl acetate: methanol. Seven different fractions were collected from the column. The elutes collected were monitored by thin layer chromatography for homogeneity and the similar fractions were pooled together. Six compounds (MPR-01, MPR-02, MPR-03, MPR-04, MPR-05 and MPR-06) were isolated from these fractions.

Spectroscopy of Isolated Compounds:

1. **MPR-01**: The IR spectrum of compound **showed** absorption bands at 3851.0 (Hydrogen bonding), 3748.9 & 3332.4 (O-H, free hydroxyl group), 2959.0 (Ali- C-H, str), 1734.9 (C=O, ester), 1614.9 (C=C stretch), 1514.3, 1463.6 and 1415.2 (C-C ring stretch), 1260.5 (C-C stretching), 1020.6 (C-O-C), 800.8 and 719.2 (monosubstituted in aromatic ring). The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 3.25 (1H, m, H-3), 4.47 (1H, m, H- 22), 3.51 (1H, m, H-3), 2.61 (1H, m, H-20), 1.33-2.15 (5H, m) ppm. Other peaks are observed at δ 0.87 (m, 9H), 1.25 (m, 5H), 1.97 (m, 4H), 2.15 (m, 5H), 0.85 (m, 3H), 1.31 (m, 9H) ppm. The FTIR and ^{1}H-NMR values of this compound were compared with reported literatures [12, 13, 14] and the mmp reading as well as Co-TLC data by comparison with authentic sample gave inference that the compound could be characterized as β-sitosterol.

2. **MPR -02**: The IR spectrum of the compound showed absorption bands at 3778.6 (Hydrogen bonding), 3691.8 to 3376.1 (O-H, free hydroxyl group), 3022.6 (Cyclic C-H, str), 2927.2 (Ali- C-H, str), 2401.1, 2337.4 and 2269.1 (Alkyne group), 1812.9 (C=O, ester), 1661.7 (C=C stretch), 1588.7, and 1434.1 (C-C ring stretch), 1218.1 (C-C stretching), 929.2 (O-H, out of plane bend), 720.9 and 673.6 (monosubstituted in aromatic ring), and 487.8 (out of plane ring C=C, bend).

3. The ^{1}H-NMR spectrum of the compound displayed the characteristic signals at δ_H 3.68 (1H, s), 6.50 (1H, m), 2.06 (3H, s), 1.90 (3H, s), 1.67 (3H, s), 3.79 (1H, m), 3.76 (1H, m), 1.23 (3H, s), 1.21 (3H, s), 1.18 (3H, s).The FTIR and ^{1}H-NMR spectral results of this compound were compared with reported literatures [13, 15] and the mmp reading as well as Co-TLC data by comparison with authentic sample gave inference that this compound could be characterized as stigmasterol.

4. **MPR -03**: The IR spectrum showed absorption bands at 3962.2 and 3905.5 (Hydrogen bonding), 3756.0 to 3236.8 (O-H, free hydroxyl group), 3021.8 (Cyclic C-H, str), 2926.2 (Ali- C-H, str), 2401.2, 2372.4, 2337.7 and 2226.3 (Alkyne group), 1724.9 (C=O, ester), 1661.4 (C=C stretch), 1589.3, 1472.3 and 1431.7 (C-C ring stretch), 1216.6 (C-C stretching), 1024.3 (C-O-C), 929.4 (O-H, out of plane bend), 763.1 and 671.9 (monosubstituted in aromatic ring), 541.7 and 486.2 (out of plane ring C=C, bend).

   The ^{1}H-NMR spectrum of the compound displayed the characteristic signals at δ_H 8.025 (d, J=1.0 Hz, H2), 7.611 (aromatic proton), 6.752 (Olefinic proton), 6.414 (d H6), 6.210 (d H8), 5.374 (d, H-1), 4.074 (m, H-2), 3.768 (OCH_3-), 3.474 (-OC(=O)Ph, t), 2.300 (CH_3CH_2-, 3H), 1.955 (s, H4), 1.280 (H, CH), 0.867 (d, CH_3). The mass data which showed m/z = 647 (100) [M^+], was an indicative of $C_{30}H_{31}O_{16}$ with other fragments 566 [647-C_6H_9]$^+$, 520 [566-C_2H_6O]$^+$, 384 [520-$C_9H_{12}O$]$^+$, 325 [384-C_3H_7O]$^+$, 295 [325-CH_2O]$^+$, 265 [295-C_2H_6]$^+$, 263 [265-H_2]$^+$.

   The UV absorption maxima at 310.5 nm were characteristic for quercetin derivatives with a galactosyl moiety. The Mass spectra revealed the [M+ H]+ ion at m/z 647conforming the molecular formula $C_{30}H_{31}O_{16}$. The ^{1}H spectra established the presence of a quercetin skeleton, a galactose unit, and a feruloyl moiety in the molecule. All the ^{1}H C signals were almost identical with those published for quercetin 3-O-(feruloyl)-β-D-galacto-pyranoside.

   The structure of this flavonol glycoside was identified on the basis of extensive spectroscopic data analysis and by comparison of spectral data with that of the related compounds reported in the literatures [16, 17]. The compound MPR-03 was

named as 2-(3, 4-dihydroxyphenyl)-5,7-dihydroxy-3-feruloyl-β-D-galactopyranosyl-4*H*-chromen-4-one.

5. **MPR-04**: The IR spectrum of compound showed absorption bands at 3388.4 (O-H, free hydroxyl group), 2932.3 (Ali- C-H, str), 1630.3 (C=C stretch), 1387.2 (C-C ring stretch), 1074.6 (C-O-C), 933.1 (O-H, out of plane bend), 779.1 (monosubstituted in aromatic ring), 620.9 (out of plane ring C=C, bend). The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 8.12 (1H, H-6'), 7.96 (1H, H-2'), 7.31 (1H, H-5'), 7.29 (1H, H-8), 7.20 (1H, H-6), 4.52 (1H, m, H-3), 3.36(1H, m, H-3), 2.54(1H, H-8), 1.24 (1H, H-6) ppm. The FTIR and ^{1}H-NMR values of this compound were compared with reported literatures [18, 19] and the mmp reading as well as Co-PC data by comparison with authentic sample gave inference that this compound could be characterized as Quercetin.

6. **MPR-05**: The IR spectrum of the compound showed absorption bands at 3366.5 to 3286.9 (O-H, free hydroxyl group), 3063.0 (Cyclic C-H, str), 1705.7 (C=O, ester), 1619.2 (C=C stretch), 1541.0, 1448.2.3 and 1339.1 (C-C ring stretch), 1245.0 (C-C stretching), 1024.3 (C-O-C), 867.1 (O-H, out of plane bend), 764.8 and 701.1 (monosubstituted in aromatic ring), 570.3 (out of plane ring C=C, bend).

   The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H 8.04 (1H, *d*, H-2), 6.96 (1H, *d*, H-5), 6.53 (1H, *d*, H-6), 7.28 (1H, H-6), 6.66 (1H, H-8), 7.12 (1H, *s*, OH-3), 6.94 (1H, *s*, OH-4), 9.21 (1H, *s*, OH-5) of aglycone; 4.40 (1H, H-1), 3.49 (1H, H-2), 3.35 (1H, *m*, H-3), 3.21 (1H, *m*, H-4), 3.26 (1H, *m*, H-5), 1.20 (3H, H-6) of rhamnose of rutinose; 5.04 (1H, H-1), 3.28 (1H, *m*, H-2), 3.49 (1H, H-3), 3.43 (1H, H-4., 3.30), (1H, *m*, H-5., 3.37 (1H, *m*, H-6), 3.51 (1H, H-6) of glucose of rutinose; 5.03 (1H, *d*, H-1), 3.54 (1H, H-2), 3.59 (1H, *m*, H-3), 3.34 (1H, *m*, H-4), 3.94 (1H, H-5), 3.93 (1H, H-6), 4.33 (1H, H-6) of glucose of 6-*O*-benzoyl glucose; 6.94 (2H, H-2, H-6), 6.66 (2H, H-3, H-5), 6.59 (1H, H-4) of benzoyl moiety. The FTIR and ^{1}H-NMR spectral results of this compound were compared with reported literature [20] and the mmp reading as well as Co-PC data by comparison with authentic sample gave inference that this compound could be characterized as rutin.

7. **MPR -06**: The IR spectrum showed absorption bands at 3874.1 (Hydrogen bonding), 3779.1, 3421.4 and 3341.3 (O-H, free hydroxyl group), 3022.0 (Cyclic C-H, str), 2924.2 (Ali- C-H, str), 2856.7 (CH_2 symmetric stretching), 2403.1, 2338.8 and 2078.4 (Alkyne group), 1603.7, 1520.5 and 1435.4 (C-C ring stretch), 1216.3 (C-C stretching), 929.0 (O-H, out of plane bend), 762.8 and 671.1 (monosubstituted in aromatic ring), 568.1 and 501.9 (out of plane ring C=C, bend).

The ^{1}H-NMR spectrum of the compound displayed the characteristic signals at δ_H 7.144 (Aromatic proton, s), 6.798 (olefinic proton, m), 6.463 (Ar-H), 3.834 (OCH_3-, s), 2.016 (OCOR), 1.250 (H, CH). The mass data which showed m/z = 871 (100) $[M^+]$ indicative of $C_{42}H_{41}O_{21}$ with other fragments 771 $[871\text{-}C_6H_{12}O]^+$, 663 $[771\text{-}C_6H_4O_2]^+$, 342 $[771\text{-}C_{16}H_{33}O_6]^+$, 295 $[342\text{-}CH_3O_2]^+$, 263 $[295\text{-}C_2H_6]^+$. The UV spectrum with maxima at 273 nm suggested a flavonoid glycoside with a sugar moiety (rhamnopyranosyl).

From the NMR spectra it exhibited signals attributed to two methyl groups, aromatic band signal substituted of feruloyl tartaric acid and Caffeoyl along with the rhamnopyranosyl moiety. The molecular formula of MPR-06 was assigned as $C_{42}H_{41}O_{21}$ on the basis of the molecular ion peak at m/z 871. The FTIR, ^{1}H-NMR and Mass values of this compound were compared with that of the related compounds reported in literatures [16, 17], which gave inference that the compound could be characterized as flavonoid glycoside containing rhamnopyranosyl and flavonoid ring substituted with feruloyl tartaric acid and Caffeoyl moiety.

The identified compound was named as 2-(3-caffeoyl-4-hydroxyphenyl)-5-hydroxy-6-rhamnopyranosyl-7-feruloyltartaric acid-4*H*-chromen-4-one.

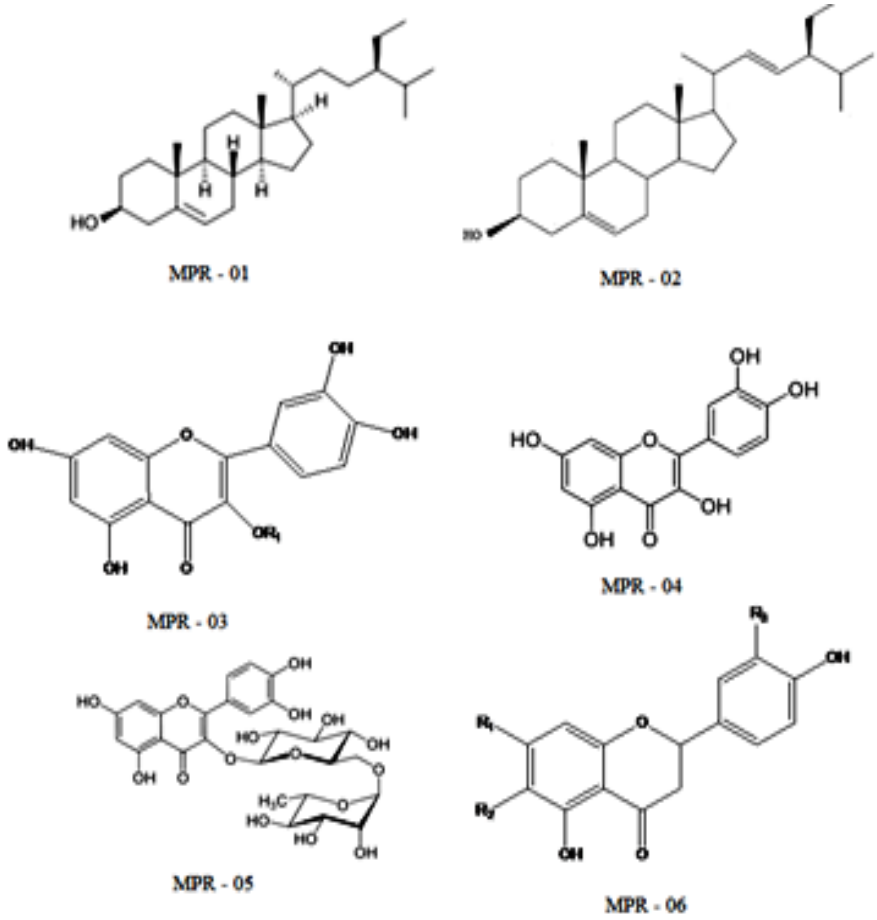

FIGURE 1: STRUCTURES OF ISOLATED COMPOUNDS FROM THE ROOTS OF *MIMOSA PUDICA* LINN.

Antimicrobial activity study of root extracts and isolated compounds (first time reported):

Standard drugs used as reference for activity studies: Chloramphenicol and Ketoconazole, two pure drugs, procured from M/s Science World, Bhopal (MP), were used as standard drugs in the present work for antibacterial and antifungal activity studies respectively.

Microorganisms used: For the present study, the bacteria and fungi used, include the gram +ve bacteria such as *Staphylococcus epidermidis* (MTCC 2639), *Staphylococcus aureus* (MTCC 7443), *Streptococcus mutans* (MTCC 890), *Streptococcus pneumoniae* (MTCC 655) and *Bacillus subtilis* (MTCC 619); gram –ve bacteria such as *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1688), *Proteus vulgaris* (MTCC 744) and *Neisseria cinerea* (MTCC 3583) and fungi such as *Aspergillus flavus* (MTCC 871) and *Candida albicans* (MTCC 227). All the MTCC strains of both bacteria and fungi were procured through M/s Growtips Biotech Research Lab., Bhopal (MP).

Preparation of stock solutions [21, 22]: Stock dilutions of the standard drug solutions of concentrations 1000 and 100 µg/L were prepared as per requirement from original stock solution (10,000 mg/L). Then two rows of 12 steriled (7.5 x1.3 cm) & capped tubes were arranged in the rack. 8 ml of broth containing required concentration of the standard drug solution (required for first tube of each row) was prepared from the existing stock solution and kept in a sterile 30 ml (Universal) screw capped bottle. The contents of the universal bottle were properly mixed and 2 ml from it was transferred to the first tube in each row.

Then, 4ml of the broth was added to the remaining 4ml content in the universal bottle by the help of a fresh pipette and and mixed well. Again, 2 ml from it was transferred to the second tube in each row.In this way, dilutions were prepared. 2 ml of antibiotic free broth was placed in the last tube in each row. One row was inoculated with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity (similarly diluted).

The result of the test was significantly affected by the size of the inoculums as the test mixture was containing 10^6 organisms / ml. Finally the tubes were incubated for 18 h at 37°C. A tube containing 2 ml broth with the organism was inoculated and kept in a refrigerator at 4°C overnight which was to be used as standard for the determination of complete inhibition.

Determination of zone of inhibition by disc diffusion method: Antimicrobial activity of *M. pudica* root extracts as well as isolated compounds (first time reported) was determined by "disc diffusion" method in reference to the reported literatures [23, 24, 25, 26]. Petri-plates containing 20 ml of agar medium were seeded with a 24 h culture of the microbial strains. The sterilized filter paper discs (Whatman no. 1) of 6mm in diameter were impregnated individually with plant extracts as well as isolated compounds at various concentrations ranging from 50 to 250 mg/ml and 5 to 200 µg/ml respectively and placed on the inoculated agar. The inoculum size was adjusted so as to deliver a final inoculum of approximately 108 colony-forming units (CFU)/ml.

Incubation was performed for both bacteria and fungus at 37°C for 24 h and 37°C for 72 h respectively. The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the disc. A standard drug, chloramphenicol was used as a positive control for comparison of antibacterial activity and ketoconazole for antifungal activity respectively. All assays tests were carried out in triplicate for each and every concentration of root extracts as well as isolated compounds (first time reported) and the results were depicted in **tables 3 and 4**.

Determination of Minimum inhibitory concentration (MIC): Dilution susceptibility testing method was used for MIC determination in reference to the cited literatures [27, 28] wherein, 75 µl of sterile nutrient broth media was decanted into each well of a sterile 96-well microplate. Highest concentration of the plant extract/isolate was added at 75 µl to the first well. After mixing of the above, 75 µl of the same was transferred to the second well and in this way, the dilution procedure was continued for the subsequent wells to attain a series of dilutions of 1/2, 1/4, 1/8, 1/32, 1/64, 1/128, 1/256, 1/512 and 1/1024 respectively. Inoculum solution at 1.5 µl was added to every well. Being incubated for 24 h at 37°C, the tubes were monitored for turbidity growth and non-turbidity as no growth. The MIC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity. Solvent blanks and positive controls were also included. All the tests were carried out in triplicate. The MIC values of all the root extracts as well as isolated compounds (first time reported) of *M. pudica* were calculated for all the used microbes (bacteria & fungi) and the results were recorded in the **tables 5 and 6**.

TABLE 3: DATA SHOWING ZONE OF INHIBITION (MM) OF *M. PUDICA* ROOT EXTRACTS AGAINST SELECTED GM. + VE AND GM. – VE BACTERIA AND FUNGI

Conc.	Gram Positive Bacteria					Gram Negative Bacteria					Fungi	
	Sta. epi.	*Sta. aur.*	*Str. Mut.*	*Str. Pneu.*	*Baci. Sub.*	*Sal. typhi*	*Esch. coli*	*Pseu. aeru.*	*Pro. vulg.*	*Neis. cine.*	*Asp. fla*	*Can. alb.*
Petroleum ether Extract												
50 mg / ml	-	-	-	-	-	-	-	-	-	-	-	-
100 mg/ ml	-	-	-	-	-	-	0.4±0.02	0.5±0.01	-	-	-	-
150 mg/ ml	-	-	1.2±0.13	-	1.2±0.26	0.6± 0.02	1.2±0.06	0.9±0.03	-	-	0.4±0.02	0.8±0.04
200 mg/ ml	0.8±0.03	1.4±0.15	1.8±0.22	0.4± 0.03	1.8±0.44	1.1± 0.08	1.2±0.05	1.6±0.16	1.2±0.03	-	1.1±0.05	1.1±0.06
250 mg/ ml	1.6±0.11	2.3±0.26	2.6±0.12	0.8± 0.02	2.6±0.19	1.2± 0.12	2.5± 0.27	1.8±0.17	1.9±0.24	-	2.3±0.13	1.7±0.11
Ethyl acetate Extract												
50 mg/ ml	-	-	-	-	-	-	4.5±0.56	2.7±0.07	0.6±0.01	-	3.1±0.54	2.4±0.05
100 mg/ ml	1.3±0.22	-	-	-	2.1±0.17	3.4± 0.42	6.2± 0.72	6.3±0.45	2.4±0.06	-	4.6±0.68	6.5±0.48
150 mg/ml	4.2±0.31	2.9±0.15	3.2±0.05	0.8± 0.02	6.3±0.32	7.2± 0.82	10.5±1.06	8.1±0.88	5.3±0.46	0.8±0.03	9.2±0.59	14.7±0.86
200 mg/ml	10.5±0.86	8.1±0.67	5.6±0.24	2.8± 0.06	9.5±0.87	11.7±1.29	14.3±1.12	13.2±1.04	12.2±0.87	1.5±0.13	17.1±1.25	19.2±1.43

250 mg/ml	19.7±1.24	11.2± 1.34	8.9±0.36	6.5± 0.23	14.7±1.57	16.5±1.34	19.1±1.56	18.2±1.25	23.1±1.68	2.6±1.21	31.5±2.96	28.3±1.38
Methanol Extract												
50 mg/ml	-	-	-	-	-	-	1.2± 0.04	0.8± 0.02	-	-	1.1±0.07	0.6±0.02
100 mg/ml	-	-	-	-	0.7±0.06	1.3± 0.08	1.9± 0.08	2.1± 0.12	0.7± 0.02	-	1.3±0.05	1.9±0.23
150 mg/ml	1.2±0.12	0.4±0.06	0.8± 0.02	0.5± 0.02	2.2± 0.19	2.4± 0.31	3.4± 0.44	3.3± 0.38	1.3± 0.03	-	2.4±0.16	4.2±0.35
200 mg/ml	3.5±0.25	3.2±0.21	2.2± 0.12	1.6± 0.12	3.7± 0.23	5.3± 0.54	3.6± 0.51	5.8± 0.64	5.4± 0.48	-	4.3±0.21	5.1±0.37
250 mg/ml	6.4±0.54	5.3±0.43	4.8± 0.31	2.7± 0.25	6.4± 0.36	5.7± 0.62	6.4± 0.69	6.2± 0.74	7.6± 0.94	0.8±0.04	7.6±0.54	6.2±0.45
Std. Drug	**Chloramphenicol**										**Ketoconazole**	
5 µg/ml	5.4±0.52	17.2±1.23	2.3± 0.02	8.9±0.13	18.1±1.26	21.3±1.78	21.2±2.24	12.3±1.42	-	7.4±0.29	9.2±0.05	18.5±1.26
25 µg/ml	12.1±0.97	21.5±1.85	5.8± 0.35	14.6±1.62	23.6±1.93	26.2±1.75	28.4±2.65	16.1±1.29	-	14.3±1.23	16.4±1.32	20.6±1.24
50 µg/ml	18.3±1.47	27.3±2.02	8.6± 0.41	22.9±2.68	28.1±2.36	32.2±2.97	33.1±2.89	25.3±1.62	6.1±0.34	23.2±1.63	22.1±1.45	31.9±1.66
100 µg/ml	21.2±1.64	30.2± 2.57	16.5±2.54	28.6±2.32	31.8±2.15	35.1±2.68	38.3±3.04	29.7±2.17	8.3±0.79	26.8±2.62	28.3±1.87	36.4±1.89
200 µg/ml	26.3±2.68	36.1±2.41	22.8±2.86	32.7±3.04	39.5±2.59	43.1±3.54	41.3±2.69	32.1±2.43	15.7±1.06	32.7±2.84	33.8±2.06	41.7±2.26

Sta.epi.- Staphylococcus epidermidis, Sta.aur.- Staphylococcus aureus, Str.Mut.- Streptococcus mutans, Str.Pneu.- Streptococcus pneumoniae, Baci.Sub.- Bacillus subtilis, Sal. typhi – Salmonella typhi, Esch. coli – Escherichia coli, Pseu.aeru.- Pseudomonas aeruginosa, Pro.vulg.- Proteus vulgaris, Neis. cine. - Neisseria cinerea, Asp.fla. – Aspergillus flavus and *Can. alb. – Candida albicans.* Mean ± SEM, n = 3. The results were the mean values of tests repeated three times after every 24 h of inhibition for bacteria and after every 72 h of inhibition for fungi at 37°C. '–': No inhibition. Results: Inhibition diameter in mm ± SEM.

TABLE 4: DATA SHOWING ZONE OF INHIBITION (MM) OF ISOLATED COMPOUNDS (FIRST TIME REPORTED) OF *M. PUDICA* ROOT EXTRACTS AGAINST SELECTED GM. + VE AND GM. – VE BACTERIA AND FUNGI

Conc.	Gram Positive Bacteria					Gram Negative Bacteria					Fungi	
	Sta. epi.	*Sta. aur.*	*Str. Mut.*	*Str. Pneu.*	*Baci. Sub.*	*Sal. typhi*	*Esch. coli*	*Pseu. aeru.*	*Pro. vulg.*	*Neis. cine.*	*Asp. fla*	*Can. alb.*
MPR - 03												
5 µg/ml	-	-	-	-	-	-	2.2±0.18	0.9±0.04	-	-	2.5±0.27	-
25 µg/ml	0.8±0.02	-	-	-	1.5±0.07	2.4±0.34	5.1±0.43	4.8±0.37	0.5±0.02	-	3.8±0.41	2.4±0.12
50 µg/ml	6.5±0.23	2.4±0.26	2.4±0.03	0.4±0.02	4.6±0.32	6.7±0.54	9.2±0.74	7.6±0.64	2.6±0.28	-	7.8±0.69	8.9±0.58
100 µg/ml	8.9±0.76	5.8±0.85	3.8±0.25	2.3±0.04	7.9±0.48	9.8±0.78	12.9±1.23	11.8±0.74	8.3±0.53	0.4±0.02	16.9±1.32	15.7±1.14
200 µg/ml	15.7±1.56	9.7±0.94	6.9±0.81	5.4±0.32	12.8±1.24	14.5±1.28	18.3±2.25	15.6±1.38	17.4±1.58	1.1±0.15	30.4±2.86	23.4±2.26
MPR - 06												
5 µg/ml	-	-	-	-	-	-	1.5±0.18	-	-	-	1.2±0.22	1.6±0.23
25 µg/ml	0.4± 0.02	-	-	-	0.8±0.06	1.8± 0.35	3.9±0.52	0.4±0.02	-	-	2.7±0.35	4.9±0.39
50 µg/ml	5.7± 0.54	2.1±0.22	1.8±0.02	-	3.9±0.38	5.9± 0.74	7.8± 0.39	2.8±0.21	1.7±0.13	-	6.9±0.53	12.3±0.78
100 µg/ml	7.8± 0.38	4.9±0.36	3.6±0.35	1.8±0.22	7.4±0.27	9.5± 0.42	11.6±0.94	6.7±0.43	6.3±0.28	-	12.4±0.68	17.6±1.32
200 µg/ml	14.9± 1.12	9.4±0.85	5.9± 0.54	4.9± 0.48	12.4±0.88	14.7±1.22	16.9±1.48	14.6±0.86	15.2±1.28	0.8± 0.05	23.9±2.45	24.5±2.52
Std.Drug	**Chloramphenicol**										**Ketoconazole**	
5 µg/ml	5.4± 0.52	17.2±1.23	2.3± 0.02	8.9± 0.13	18.1±1.26	21.3±1.78	21.2±2.24	12.3±1.42	-	7.4± 0.29	9.2± 0.05	18.5±1.26
25 µg/ml	12.1±0.97	21.5±1.85	5.8± 0.35	14.6±1.62	23.6±1.93	26.2±1.75	28.4±2.65	16.1±1.29	-	14.3±1.23	16.4±1.32	20.6±1.24
50 µg/ml	18.3±1.47	27.3±2.02	8.6± 0.41	22.9±2.68	28.1±2.36	32.2±2.97	33.1±2.89	25.3±1.62	6.1±0.34	23.2±1.63	22.1±1.45	31.9±1.66
100 µg/ml	21.2±1.64	30.2±2.57	16.5±2.54	28.6±2.32	31.8±2.15	35.1±2.68	38.3±3.04	29.7±2.17	8.3±0.79	26.8±2.62	28.3±1.87	36.4±1.89
200 µg/ml	26.3±2.68	36.1±2.41	22.8±2.86	32.7±3.04	39.5±2.59	43.1±3.54	41.3±2.69	32.1±2.43	15.7±1.06	32.7±2.84	33.8±2.06	41.7±2.26

Sta.epi.- Staphylococcus epidermidis, Sta.aur.- Staphylococcus aureus, Str.Mut.- Streptococcus mutans, Str.Pneu.- Streptococcus pneumoniae, Baci.Sub.- Bacillus subtilis, Sal. typhi – Salmonella typhi, Esch. coli – Escherichia coli, Pseu.aeru.- Pseudomonas aeruginosa, Pro.vulg.- Proteus vulgaris, Neis. cine.- Neisseria cinerea, Asp.fla. – Aspergillus flavus and *Can. alb. – Candida albicans.* Mean ± SEM, n = 3. The results were the mean values of tests repeated three times after every 24 h of inhibition for bacteria and after every 72 h of inhibition for fungi at 37°C. '–': No inhibition. Results: Inhibition diameter in mm ± SEM. MPR-03 & MPR-06: Isolated compounds (first time reported)

TABLE 5: DATA SHOWING MINIMUM INHIBITORY CONCENTRATION (MG/ML) OF *M. PUDICA* ROOT EXTRACTS AGAINST SELECTED GM. + VE AND GM. – VE BACTERIA AND FUNGI

Organisms		P.E. Ext.	E.A. Ext.	M. Ext.
Gram Positive Bacteria	*Staphylococcus epidermidis*	25	3.12	18.75
	Staphylococcus aureus	12.5	37.5	37.5
	Streptococcus mutans	9.37	4.68	18.75
	Streptococcus pneumoniae	12.5	18.75	18.75
	Bacillus subtilis	18.75	25	25
Gram Negative Bacteria	*Salmonella typhi*	37.5	12.5	25
	Escherichia coli	50	3.12	12.5
	Pseudomonas aeruginosa	12.5	6.25	12.5
	Proteus vulgaris	12.5	0.39	25
	Neisseria cinerea	-	18.75	31.25
Fungi	*Aspergillus flavus*	75	0.39	12.5
	Candida albicans	37.5	1.56	12.5

P.E. Ext. – Petroleum ether extract, E.A. Ext. – Ethyl acetate extract and M. Ext. – Methanol extract

TABLE 6: DATA SHOWING MINIMUM INHIBITORY CONCENTRATION (µG/ML) OF ISOLATED COMPOUNDS (FIRST TIME REPORTED) FROM *M. PUDICA* ROOT EXTRACT AGAINST GM. + VE AND GM. – VE BACTERIA AND FUNGI

Organisms		MPR-03	MPR-06
Gram Positive Bacteria	*Staphylococcus epidermidis*	6.25	3.12
	Staphylococcus aureus	3.12	6.25
	Streptococcus mutans	6.25	3.12
	Streptococcus pneumoniae	12.5	12.5
	Bacillus subtilis	3.12	6.25
Gram Negative Bacteria	*Salmonella typhi*	3.12	6.25
	Escherichia coli	1.25	0.62
	Pseudomonas aeruginosa	1.25	12.5
	Proteus vulgaris	6.25	6.25
	Neisseria cinerea	12.5	50
Fungi	*Aspergillus flavus*	1.25	1.25
	Candida albicans	1.5	1.25

MPR-03 & MPR-06: Isolated compounds (first time reported)

RESULTS AND DISCUSSION: In the present study, preliminary phytochemical screening of various solvent extracts of roots of *M. pudica* were carried out which showed presence of biologically important secondary metabolites, such as, alkaloids, steroids, phenolic compounds, tannins, flavonoids, triterpenoids, carbohydrates, cardiac glycosides and amino acids. The ethyl acetate root extract was found to have positive tests for phenolic compounds, tannins, flavonoids and triterpenoids (Table-2). In reference to earlier reported literatures, the above metabolites were proven to have antimicrobial properties [29, 30, 31, 32, 33].

The ethyl acetate root extract (EAE) exhibited better inhibition of bacteria and fungi in comparison to other two extract (petroleum ether and methanol). EAE in a concentration of 250 mg/ ml was found to show an inhibition of 23.1 mm against the gram negative bacteria, *Proteus vulgaris*, which was even more than that of Chloramphenicol (standard drug) in a concentration of 200 µg/ ml (15.7 mm). Likewise, the EAE in a concentration of 250 mg/ ml showed an inhibition of 31.5 mm against the fungi, *Aspergillus flavus*, which was well comparable with that of 33.8 mm inhibition of Ketoconazole (standard drug) in a concentration of 200 µg/ ml (Table 3).

The lowest MIC of 3.12 mg/ ml was observed in EAE against the gram positive bacteria, *Staphylococcus epidermidis* and the gram negative bacteria, *Escherichia coli* and 0.39 mg/ml of EAE against *Aspegillus flavus* (Table – 5).

The isolated compound, MPR-03 in the concentration of 200 µg/ ml showed better inhibition of gram positive bacteria (15.7 mm against *Staphylococcus epidermidis*), gram negative bacteria (18.3 mm against *Escherichia coli*) and fungi (30.4 mm against *Aspergillus flavus*) while comparing with that of MPR-06. Particularly in case of inhibition of fungi, 200 µg/ ml concentration of MPR-03 was well comparable and even more susceptible in comparison to 100 µg/ ml of the standard drug, Ketoconazole (Table – 4).

The lowest MIC of 3.12 µg/ ml was noticed in both MPR-03 and MPR-06 against the gram positive bacteria (MPR-03 against both *Staphylococcus aureus* and *Bacillus subtilis* whereas, MPR-06 against both *Staphylococcus epidermidis* and *Streptococcus mutans*). But the lowest MIC of 0.62 µg/ ml was only found in MPR-06 against the gram negative bacteria, *Escherichia coli*. In case of inhibition of fungi, the lowest MIC of 1.25 µg/ ml was noticed in both MPR-03 and MPR-06 against both *Aspergillus flavus* and *Candida albicans* respectively (Table – 6).

CONCLUSION: The systemic screening of plant extracts as well as isolates for antimicrobial activity is a continuous effort to explore new antimicrobial compounds. Considering the rich diversity of medicinal plants in Odisha, it is worthwhile to screen plants/plant parts for their inherent antimicrobial activities which can substitute synthetic medicinal compounds, being extensively used now-a-days.

From the present studies, it was concluded that the traditional plants might represent new sources of anti-microbial agents with biologically active and stable components which could establish a scientific base for the use of plant metabolites in modern medicines for ailment of various microbial infections.

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