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Research Article

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Chemical Investigation of Seed of *Ipomoea hederacea* and its Biological Activity

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ABSTRACT

Ipomoea is the largest genus in the flowering plant family "Convolvulaceae" with over 500 species, most of these are called morning glories. Ipomoea hederacea considered as a valuable herb for indigenous medicine is renowned for its medicinal properties. Ipomoea hederacea is a favored drug for different diseases because of its oral effectiveness, good safety profile availability in India. The seed of this plant have also been used in both analgesic and antiseptic properties. The present work is aim to investigate the phytochemical investigations of different extracts of seed of Ipomoea hederacea. The methanolic extract of seed was directly chromatographed over silica gel column eluted with solvent petroleum ether, chloroform, acetone and methanol to their increasing order of polarity. The various extracts were directly separated by column chromatography and monitored by thin layer chromatography. The extracts were characterized on the basis of various spectral techniques such as IR, ^{1}H NMR, and mass spectroscopy. The antibacterial study performed against to bacteria species via; Escherichia coli and pseudomonas aeruginosa. The methanol extracts of seed of Ipomoea hederacea exhibited varying level of antibacterial activity, with minimum inhibitory concentration (MIC) of 2 mg/ml against both bacteria. The methanol extract was found to be more active than the other extract like petroleum ether, chloroform and acetone. The antifungal activity of these extracts was also performed against to alternaria brassica, alternaria braceacola, aspergillus Niger fungal strain. The methanol and chloroform extracts shows moderated as well as significant activity against the strains.

Keywords: Ipomoea Hederacea, Biological activity, Seed, Herbicides, Diabetes.

INTRODUCTION

India is a home to abundant medicinal plant and animal life and has a wide range of climates that accommodate a diversity of species throughout the country. A large number of medicinal plants are present in hill region which are known but these have not been investigated chemically yet and put to use in modern system of medicine. Herbicides have been effective in reducing the effect of weeds on crop yield for more than 50% [1]. Moreover recommended rates of herbicides are often higher than what is actually needed for effective weed control. There are about 20,000 species of tropical plant in Mexico out of which 5,000 are considered to possess medicinal values, which is yet to be documented and evaluated by means of scientific approaches [2]. Medicinal herb is considered to be a chemical factory as it contains multitude herbs are using in the treatment of heart disease, skin disease, high blood pressure, pain, asthma and other problems. Ipomoea hederacea is commonly known as Ivy leaf morning glory, pharbits seeds and kaladana. The most important species Ipomoea batatas extensively cultivated for its edible root tubers. A number of species have been introduced in India and many species are grown in gardens for ornamental purposes, some are of medicinal value [3,4]. Leaf wax contains primarily a variety of short and long chain hydrocarbons, alcohols, acids, esters, aldehydes and triterpenes. Morning glory leaves are easily removed with defoliants or disicants, but stems or tough and wiry causing them to be more resistant to drying. The seeds were found to have both analgesic and antiseptic properties. Chemists identified indolizidine alkaloids in the seeds, to which the analagesic properties had attributed. Different formulation of the crude drug has been made, the treatment of skin

ailments, glycerol preparation for the treatment of pharyngitis. One of the species of Ipomoea is used in treatment of Diabetes [5]. Friedman et al [6] studied several varieties of Ipomoea seeds which contain ergot alkaloid and chlorogenic acid measured by a spectrophotometer procedure. Monk et al [7] synthesized imidazolinone herbicides which were used for injury currently available common field corn cultivars. Sengupta and Gupta [8] analyzed resin content from five samples of Kaladana which was supposed to be the active constituent of the drug. Hildebert and co-workers reported [9] the presence of various resins from Ipomoea. Wen-qun et al [10] reported the essential amino acid for human body in the seed of Ipomoea. Ipomoea nil is higher in nutritive value than other plants have reported by Sharma and Sharma [11]. Isoscopoletin and esculetin were separated from Ipomoea Pes-caprae sweet for the first time by the Quingji et al [12]. Benjamin and Adelia [13] reported the biochemical and nutritional compound of the sweet potato with emphasis on its β-carotene and anthocyanin contents. N-cis-feruloyltyramine-1, N-p-hydroxy- trans- cinnamoyltyramine-2, N-trans- feruloyltyramine-3, N-p-hydroxy- cis-cinnamoyltyramine-4, compounds were first isolated from Ipomoea by Lan et al [14]. Five different genotypic cultivars of Ipomoea aquatica were cultivated by Ying et al [15] to investigate their accumulation variation of di-Bu phthalate (DBP). A potato starch product was prepared [16] by processing fresh Radix Ipomoea to powder soaking fresh folium bambusae in ethanol for 7-15 days extracting, separating, vaccum concentrating to get juice by mixing the powder and juice, stirring and making succus bambusae rice noodle, succus bambusae vermicelli or succus Bambusae sheet jelly. Ming [17] composed a health food, yanshuai soft capsule of principal drug including Bulbus Allii, Radix Astragali, Radix Raphani and Radix Ipomoea. Xingbu [18] synthesized a chinese medicinal composition, shengya oral liquid, comprises cortex Moutan, Rhizoma Cimicifugae, Radix Peucedani, fructus Schisandrae and Radix Astragali. Xiachen [19] composed a composition for the treatment of epilepsy comprises two group materials. The first group comprises Lapis Chloriti, Haematitum Pumex, Lignum, Aquilariae, Resinatum and the second group comprises Scorpio Gypsum, Fibrosum, Cinabaris, Succinum. A method of eliminating dermis speckle comprises physical treatment (with laser processing, laser killing fungus activating cortical cell), APS pigment decomposition on mottle and medication treatment was discovered by Zhiqing [20]. Guoqiang [21] prepared an anti aging soup from Calocasia Esculenta, Dioscorea Opposita, Ipomoea batatas and small glue puddings. Junchaun [22] investigated a method for preparing vinegar capable of treating hyperlipidemia, hypertension, vascular sclerosis and bonespor. Some species of the genus Ipomoea such as Ipomoea tri color, Ipomoea batata and Ipomoea murucoides are traditionally used as nutrcional , emetic, diuretic, diaphoretic, purgative and pesticidal agents were described by Vera et al [23]. Hepatoprotectivity and an antioxidant study of Ipomoea Hederacea were discovered by Devi et al [24].

In the above point of view we have isolated various extracts such as chloroform extracts (C1), acetone extract (C2), and methanol extract (C3) by chromatographic techniques and the extracts were characterized by IR, ^{1}H NMR and Mass spectroscopy. The microbial activity of these extracts has been studied against bacterial and fungal strain.

EXPERIMENTAL SECTION

Plant Materials

The seeds of Ipomoea Hederacea were collected from local market, Kanpur, INDIA. The seed was identified by Dr. A. K. Kushwaha, PPN College, Kanpur. Seeds of the plant were cleaned with distilled water, dried and crushed in mixer grinder and the grinding was performed in a hygienic condition.

Extract Preparation

The powder material of seed was dissolved in methanol in a jar. The ratio of the methanol and powdered seed were 3:1, respectively. It was stirred about one hour to make sure that the compound gets dissolved in solvent and it was left for 24 hours. The extract was taken and filtered using sterile filter paper and concentrated under reduced pressure for crude products. A gummy solid was directly chromatographed over silica gel column and eluted with solvent petroleum ether, chloroform, acetone and methanol in their increasing order of polarity.

Analysis and Measurements

Spectral Analysis

The extracts were characterized by the various spectral techniques such as IR, ^{1}H NMR and mass spectrophotometer. The infra red spectra were recorded on FT-IR spectrophotometer, brucker (vertex 70) using KBr pallets in a range of 4000-400cm^{-1}. Zeol 400 MHz spectrophotometer was used for recording the ^{1}H NMR spectra using CD_3OD as solvent and TMS as internal standard. Mass spectra were recorded on a Q-Top Premier mass spectrophotometer.

Test Organisms

The test microorganism used for the antibacterial activity was performed on broth and nutrient agar media which contains 0.5g peptone, 0.5g NaCl, 0.3g beef powder extract and 1g peptone, 1g NaCl, 0.6g beef powder extract, 4.0g

agar, respectively. Bacteria were cultured over night at 28^0C for 24 hour in Muller Hington broth inoculum. Sterile Petri disc with a diameter of 6mm plates were prepared by pipetting 100µL volume of stock solution of extract (2mg/ml) on to sterile blank plates. The plates were air dried and stocked solution at 4^0C, used within two days, a plate containing solvent extract was applied to incubated plates by using flamed forcipes.

Antibacterial activity of extracts of seed of Ipomoea hederacea were evaluated by plate method using 100µL of suspension containing 10^8 CFU/ml of bacteria spread on Mullar Hington Agar medium. The extracts were dissolved in CH_3OH at a concentration of 50mg/ml. The disc impregnated with 100µL of extracts placed on seeded agar and the disc plates were incubated at 28^0C for 24 hours depending on the diameter of zone inhibition formed around the plates.

The fungi were isolated from the infected part of their respective hosts via Alternaria brassica, culture from laboratory strain. Fungi were cultured on potato dextrose agar (PDA) medium at 25 ± 2^0C, the culture were purified by single spore germination on PDA slant with composition of 250g peeled potato, 20g dextrose, 20g agar, 100 ml distilled water, slants and stored at 4^0C for further use. The purified compounds in respective solvent were tested for their antifungal activity by silica gel TLC method. Different concentration of compounds was prepared. 20µL of each concentration was spotted on TLC plates, dried at room temperature and overspread with spore suspension (1 x 10^7 spore/ml) of test fungi in Czapak –Dox medium. The plates were incubated in humid chamber at 25 ± 2^0C for 3 days until the growth of the fungus become visible. A control plates spotted with the corresponding organic solvent was run in parallel. The minimum inhibitory concentration (MIC) was defined as the minimum concentration at which on fungal growth was observed that is showing a clear zone of inhibition.

RESULTS AND DISCUSSION

IR Spectra

The IR spectral data of the extracts are shown in **Table** 1. The IR spectrum (**Figure 1**) of extract C_1 exhibited a band at 3363cm^{-1} which clearly verified the presence the hydroxyl group. One strong peak at 2925cm^{-1} indicated the presence of ν(CH_2) group. The appearance of absorption of frequency at 1700cm^{-1} showed the presence of carbonyl group.

The band at 3387cm^{-1} indicated the presence of hydroxyl group in the IR spectrum (**Figure 1**) of extract C2. The peak at 2928cm^{-1} indicated the presence of ν(CH_2) group. The ν(CO) group was confirmed due the appearance of absorption of frequencies at 1702cm^{-1}.

The appearance of peak at 3456cm^{-1} in the IR spectrum (**Figure 1**) of C_3 extract indicated the presence of ν(OH) group [25]. The appearance of absorption of frequency at 2925cm^{-1} showed the presence of ν(CH_2) group. The carbonyl group was confirmed by the presence of peaks at 1740cm^{-1}.

Table 1: IR bands (cm^{-1}) of the extracts

Extracts	υ(-OH)	υ (-CH)	υ (-CO)	υ (-COOH)
C_1	3363	2925	1700	1602
C_2	3387	2928	1702	1446
C_3	3456	2925	1740	1464

NMR Spectra

^{1}H NMR spectra (**Figure 2**) of the extract C1 showed a singlet peaks at 1.25ppm and 1.69ppm indicates the proton attach with the methyl group. The ring proton was confirmed by the appearance of the peak at 2.06ppm. The appearance of the peak at 3.67ppm indicated the presence of methylene group and appearance of peak at 4.75ppm indicated the presence of olefinic group.

The ^{1}H NMR spectrum (**Figure 2**) of C2 extract exhibited a peak at 1.25ppm indicates the presence of methyl group. The ring proton was confirmed by the appearance of the peaks at 2.03ppm to 208ppm. The appearance of the mutiplet at 7.33ppm confirmed the aromatic ring and the peak at 9.39ppm showed the presence of ketone group. Two doublet peaks at 4.5ppm and 4.7ppm showed the presence of olefinic group.

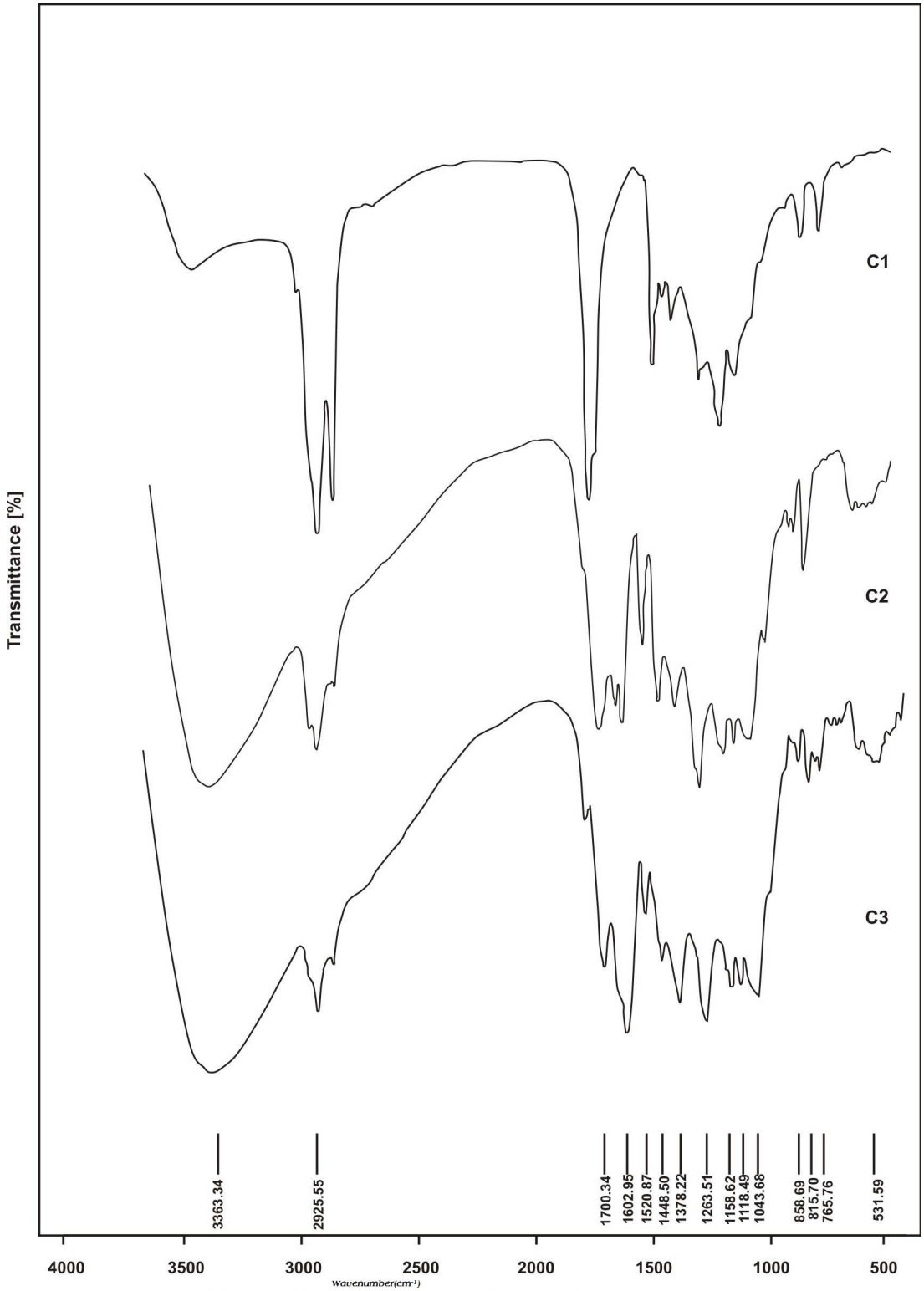

Figure 1: IR spectra of extracts C1, C2 and C3

In the ^{1}H NMR spectrum (**Figure 2**) of the extract C3, the presence of singlet peak at 0.8ppm and triplet peak at 1.17ppm confirmed the presence of methyl group in different positions. The peak due to benzene ring proton was obtained in the form of multiplet at 7.39ppm, where as peak at 3.51ppm was attributed for the ester group. The singlet and triplet peak at 5.20 ppm and 11.8 ppm showed the presence of olefinic and methylene group, respectively. The NMR data are given in **Table 2**.

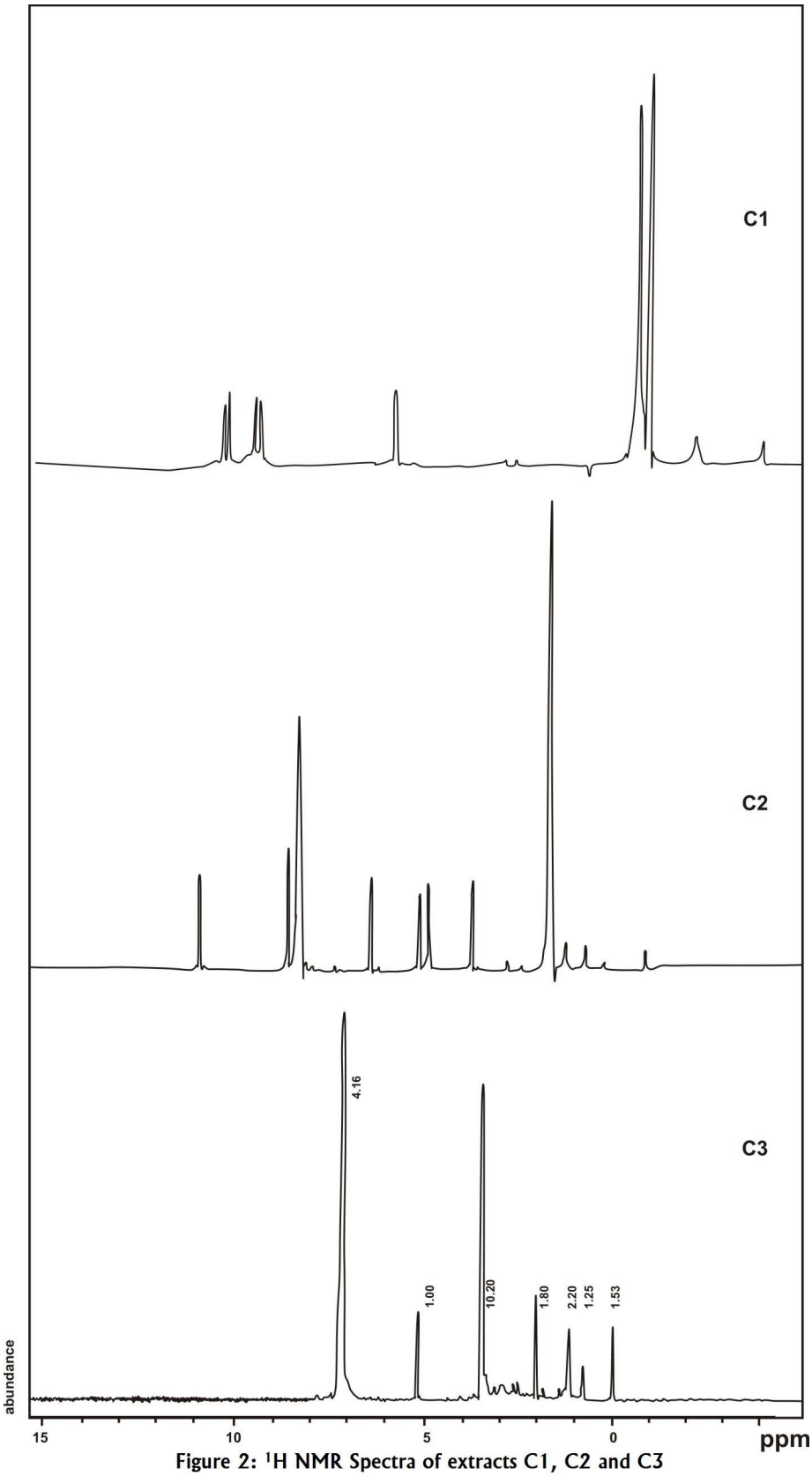

Figure 2: ^{1}H NMR Spectra of extracts C1, C2 and C3

Mass Spectra

The mass spectrum of extract C1 showed the highest molecular ion peak at m/z value 468 which was confirmed the molecular weight of the extract C1.The mass spectrum of the extract C2 showed the highest molecular ion peak at

m/z value 741 which corresponds to the molecular weight of the extract C2. The mass spectrum of extract C3 showed that the m/z value 535 which confirmed the molecular weight of the extract C3.

Table 2: ^{1}H NMR data of extracts

Extracts	^{1}H NMR data
C_1	1.25 and 1.69 (-CH_3); (s, 3H); 2.06(m, 20H); 4.56(m, 2H,-C=C-); 4.75 (d,2H).
C_2	1.25 (-CH_2-); (s, 2H); 2.03-2.08 (m, 22H); 4.5(d, 2H); 4.7(d, 1H); 7.33(m, 15H); 7.5 (s, 1H); 9.39(s, 1H, -OH).
C_3	0.8 (s, 1H); 2.54 (s, 8H); 3.51 (d, 8H); 5.20 (s, 2H,-C=C-); 7.39 (m, 6H, -OH).

Biological Studies

The in vitro antimicrobial activity of the extracts on selected bacteria and virus were carried out.

Antibacterial activity

The extracts of chloroform, methanol and acetone were performed against Escherichia coli, pseudomonas aeruginosa are given in **Table 3**. The methanol and acetone extracts were observed inhibitory against Escherichia coli and Peudomonas aeruginosa while the chloroform extract was found to inhibitory against Escherichia coli rather than pseudomonas aeruginosa.

Table 3: Antibacterial activity of extracts

Bacterial species	Chloroform extract	Acetone extract	Methanol extract
Escherichia coli	+	+	+
Pseudomonas auruginosa	-	+	+

Antifungal activity

The sensitivity of fungal strains is shown in **Table 4.** The sensitivity of various extracts revealed that the seeds extract in the acetone was inhibitory to the test organisms Alternaria brassicea, Alternaria braceacola and Aspergillus Niger. The methanol extract showed maximum sensitivity in comparison to chloroform extracts against Alternaria brassicea, Alternaria braceacola and Aspergillus Niger.

Table 4: Antifungal activity of extracts

Fungal species	Chloroform extract	Acetone extract	Methanol extract
Alternaria brassicea	+	-	+
Alternaria braceacola	-	-	+
Aspergillus niger	+	+	+

CONCLUSION

The chemical constituents like saponins, alkaloids and carbohydrates are responsible to antimicrobial activity of the crude drug. The presence of these bioactive components in the crude drugs has been linked to their activities against diseases causing microorganism and also offering the plants themselves protection against infection by pathogenic microorganism.

The extraction of biological active compounds from the plant materials depends of the type of solvent used in the extraction procedure. Most of the antimicrobial active compounds that have been identified were soluble in polar solvents. The methanol extracts of Ipomoea hederacea seed was chromatographed over silica gel column eluting with different organic solvents with their increasing order of polarity to separate the components in each solvent for their antimicrobial activity. In the IR spectra of extracts of chloroform, acetone and methanol, the functional groups ν(OH), ν(CH_2), ν(CO) and ν(COOH) were identified. The ^{1}HNMR study supported the structure regarding the presence of different protons of methylene ring proton, olefinic group and hydroxyl group. The molecular weight of the extracts was calculated with the help of mass spectra to correlate the structures which were exactly match with the proposed structures (**Figure 3, 4 & 5**). The antimicrobial study showed that the extracts were biological active against both bacteria and fungi. The methanol extracts were found to be more active than the other extracts.

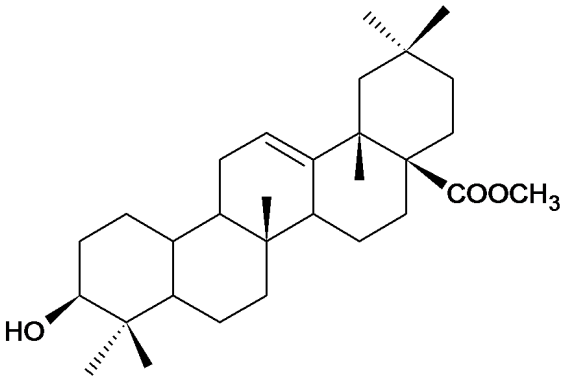

Figure 3: Proposed structure of extract C1

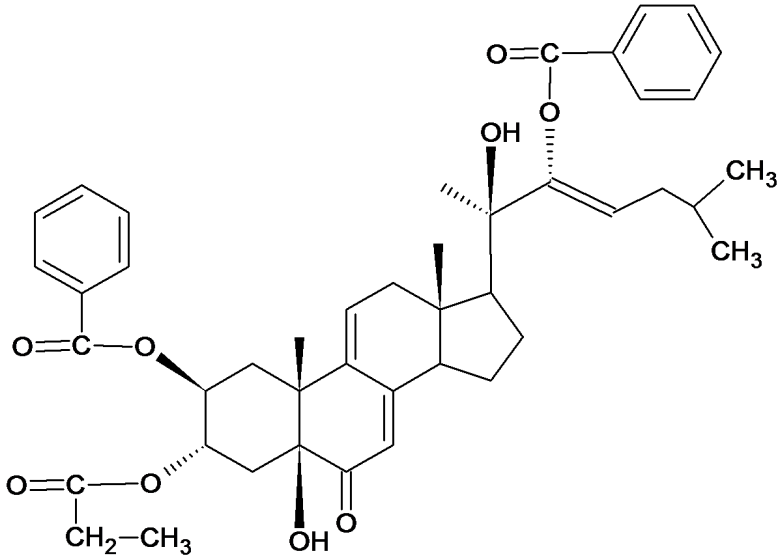

Figure 4: Proposed structure of extract C2

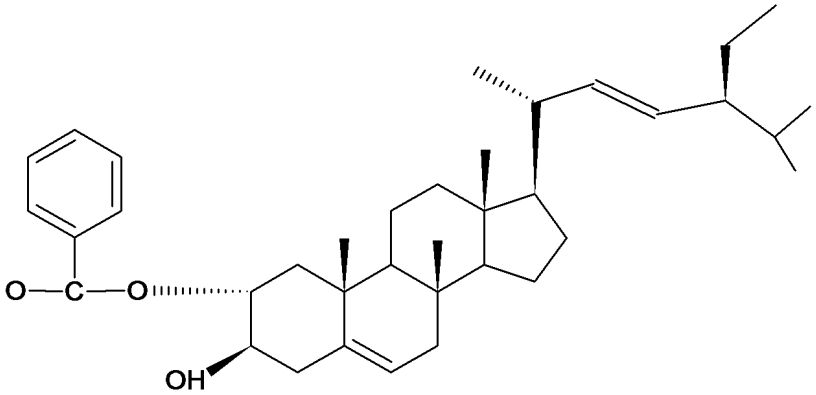

Figure 5: Proposed structure of extract C3

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