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A study on Phytochemical Screening and Characterisation of Acetone Extracts of plant leaf *Glycosmis pentaphylla*

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ABSTRACT

Glycosmis pentaphylla is one of the medicinal plant commonly known as 'panal'. Traditonally, leaves of this plant were used in the treatment of cough, rheumatism, anaemia, jaundice, ascariasis ,fever , liver complaints, eczema, skin affections etc.In the present study, acetone extracts of plant leaves were subjected to photochemical screening, HPLC and GC-MS analysis. The phytochemical screening of acetone extracts of *Glycosmis pentaphylla* has shown the presence of alkaloids, flavanoids, phenols, saponin,steroids, tannins and carbohydrates. GC-MS chromatogram of acetone extract of *Glycosmis pentaphylla* revealed the presence of 36 phyto compounds including Methanone, (4-bromo-3,5 dimethyl phenol), Propanenitrile,2,2-dimethyl-azobis,3,7,11,15-Tetramethyl-2-hexane-1-ol,gamma.-Sitosterol,Caryophyllene etc. HPLC study revealed the presence of 18 phytocompounds. These photochemical exhibit medicinal as well as pesticidal property. Therefore, it is recommended that active ingredients from *Glycosmis pentaphylla* could be used for the preparation of herbal insecticidal formulation to the control of stored grain pest, so it could be integrated into pest management system.

KEYWORDS: *Glycosmis pentaphylla*, acetone extract, GC-MS analysis, HPLC, phytocompounds.

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INTRODUCTION

Medicinal plants are gifts of nature to cure limitless number of diseases of human beings [1.] The abundance of plants on the earth's surface has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants, as potential source of new antimicrobial agents [2.] Hence, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drug against microbial infections [3.] In recent years, secondary plant metabolites, photochemical, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[4.]

According to world health organization (WHO), greater than 80% of the total world's population depends on the traditional medicines in order to satisfy their primary health care needs. It also suggested in improving the technologies for cultivation of medicinal plants [5]. The chemical substances of the medicinal plants which have the capacity of exerting various physiological actions on the human body were the primary features. Plants are able to produce a large number of diverse bioactive compounds such as alkaloids, flavonoids, tannins, phenols etc. The photochemical research that has been done based on the ethno-pharmacological information leads to the effective approach in the discovery of new anti-infective agents from higher plants [6]. These photochemical have been used as medicines, foods, cosmetics, dyes, pesticides etc.Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, ant diarrheal, analgesic and wound healing activity were reported.

Bio pesticides are made from naturally occurring substances that controls pests by non-toxic mechanisms and in ecofriendly manner. They are alternative to synthetic pesticides and for several decades plants have been a source of traditional medicine with least toxicity to mankind and domesticated animals [7].Higher plants are a rich source of novel insecticides [8]. Plant based materials, whole plants or their extracts have been potentially used for killing and repelling mosquitoes and other nuisance vectors [9].Plant extracts have been found to be useful and effective as larvicides, repellent or deterrent agents, oviposition attractants or repellent and insect growth hormone regulators [10].

Glycosmis pentaphylla (Family: Rutaceae) is an evergreen shrub commonly called an orange berry grown in eastern Asia in Bangladesh, India, and Thailand. It is used for the treatment of, cough jaundice, boils, chest pain, hookworm infestation and urinary tract infections in human.Juice extracted from *G. pentaphylla* leaves is used for treatment of fever and liver problems [11]

The present study was conducted to evaluate the photochemical compounds present in the acetone extract of the plant leaves of *Glycosmis pentaphylla*

MATERIALS AND METHODS

Materials

Preparation of Acetone extract

Acetone extract was prepared using Soxhlet apparatus.50 gm of leaf powder were weighed and tied in a thin cloth and placed in the extraction tube. 500 ml acetone was taken in the glass flask. Acetone was boiled at 55ºC continuously. Boiling was continued for 6-8 hours till the extract became pale green. After the extraction is over the extract is allowed to cool, and stored in air tight containers for further use under refrigerated condition. The acetone extract obtained was treated as 100% concentration.

Methods

Phytochemical studies

Preliminary phytochemical analysis

Acetone extact of the plant leaves of *Glycosmis pentaphylla* were used for qualitative confirmation of major photochemical constituents such as alkaloids, flavonoids, phenolics, saponins, steroids, tannins, and carbohydrates

Alkaloids

In a test tube containing 1ml of extract, a few drops of Dragendorff's reagent was added and colour development was noticed. Appearance of orange colour indicates the presence of alkaloids.

Flavonoids

5ml of 1% hydrochloric acids extract was shaken with sodium hydroxide, a yellow colour appeared indicate the presence of flavonoids.

Phenolics

1ml of extract was added in 2ml of distilled water and a few drops of 10% ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Saponins

One ml of the plant was boiled with 10 ml of water for a few minutes and filtered. The filtrate was vigorously shaken. The persistent froth (1cm height) was present for 1h which indicates the presence of saponins.

Steroids

The power was dissolved in 2ml of chloroform in a dry test tube. Ten drops of acetic anhydride and 2drops of concentrated sulphuric acid were added. The solution turning red followed by blue and finally bluish colour this indicates the presence of steroids.

Tannins

One drops of ferric chloride was added to 2ml of the extract, and the appearance of bluish or greenish black coloration indicates the presence of tannins.

Carbohydrate

In a test tube, 5ml of the filtrate was treated with 5ml of fehling's solutions (A&B) and heated. The appearance of a red precipitate indicates the presence of reducing sugars.

HPLC

HPLC is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. A waters Inc HPLC system consisting of 2695 Alliance Separation Module and model 2487 Tunable UV detector was used for the study. 20 µL of the samples were injected onto the C18 symmetry column 4.6X250 mm. The separation was effected using water containing 0.1% Formic acid as a mobile phase and the detection wave lengths were set at 275 and 236 nm respectively. HPLC of acetone fractions was carried out in a Waters Inc HPLC system. A detection wave length was set as 280 nm 4nm.

GC-MS Studies

The GC-MS of the acetone extract of the plant leaves of *Glycosmis pentaphylla* was carried out in GC-MS Varian Saturn 2200 with factor four V.F ms column and GC-MS solution software 5.2.Programme temperature was initially set as 100^0c for 1.5 minutes and gradually raised to 270^0c for 60 minutes.1 µl sample was injected for analysis. Helium gas of 99.99% purity was used as carrier gas. The flow rate of gas was set as 1 ml/min. The sample injector temperature was maintained at 250^0 c and the ionization mass was done with 70eV.Mass spectra were recorded for the mass range 40-600 m/z for about 60 min. Identification of compounds was based on comparison of the mass spectra with library as the compound separated, on dilution through the column, were detected in electronic signals.

OBSERVATION AND RESULT

Preliminary phytochemical constituents of acetone extract of Glycosmis pentaphylla

Secondary metabolites	Acetone extract
Alkaloids	+
Flavanoids	+
Phenols	+
Saponin	+
Steroids	+
Tannin	+
Carbohydrates	+

+ Present

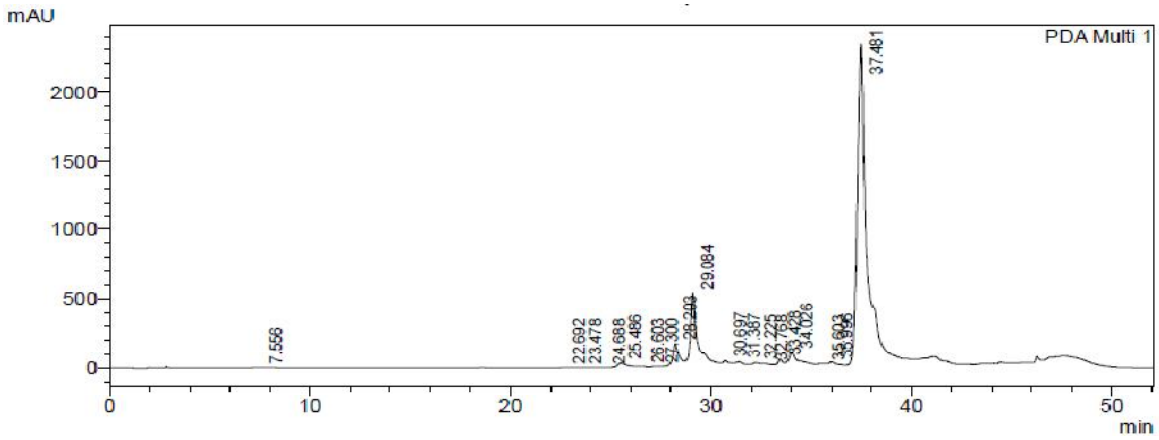

Figure 1 .Chromatogram of HPLC at 280nm 4nm of acetone extract of the plant leaves of *Glycosmis pentaphylla*

Table 1.Compounds analysed in HPLC

SI no	RT	Peak name	Area	Height	Area %	Height %
1	7.556	M-Methyl-N-n-butyltrifluoroacetamide	398379	5287	0.400	0.161
2	22.692	2-Piperidinecarboxylic acid	21129	933	0.021	0.028
3	23.478	Methionine	139596	3096	0.140	0.094
4	24.688	N-acetyl-glutamic acid	20269	1111	0.020	0.034
5	25.486	1,2,3-Trioxybenzene	1125132	30698	1.130	0.935
6	26.603	Glutamic acid	60627	3584	0.061	0.109
7	27.300	Pyrophosphate	84881	3235	0.085	0.099
8	28.203	1,4-Butanediamine (Putrescine)	3797563	164350	3.813	5.005
9	29.084	Phospho-propylester	15847658	530804	15.913	16.163
10	30.697	Tetradecanoic acid	291722	19091	0.293	0.581
11	31.387	Tyrosine	276372	16116	0.278	0.491
12	32.225	Galactose / Glucose	311186	12448	0.312	0.379
13	32.768	Pentadecanoic acid	69329	5141	0.070	0.157
14	33.428	Palmitelaidic acid	871566	47862	0.875	1.457
15	34.026	D-Gluconic acid	3231542	97277	3.245	2.962
16	35.603	Palmitic acid	370810	12390	0.372	0.377
17	35.996	Oleic acid	516268	23135	0.518	0.704
18	37.481	11-cis-Octadecenoic acid	72153651	2307435	72.452	70.263
Total			99587681	3283994	100.000	100.000

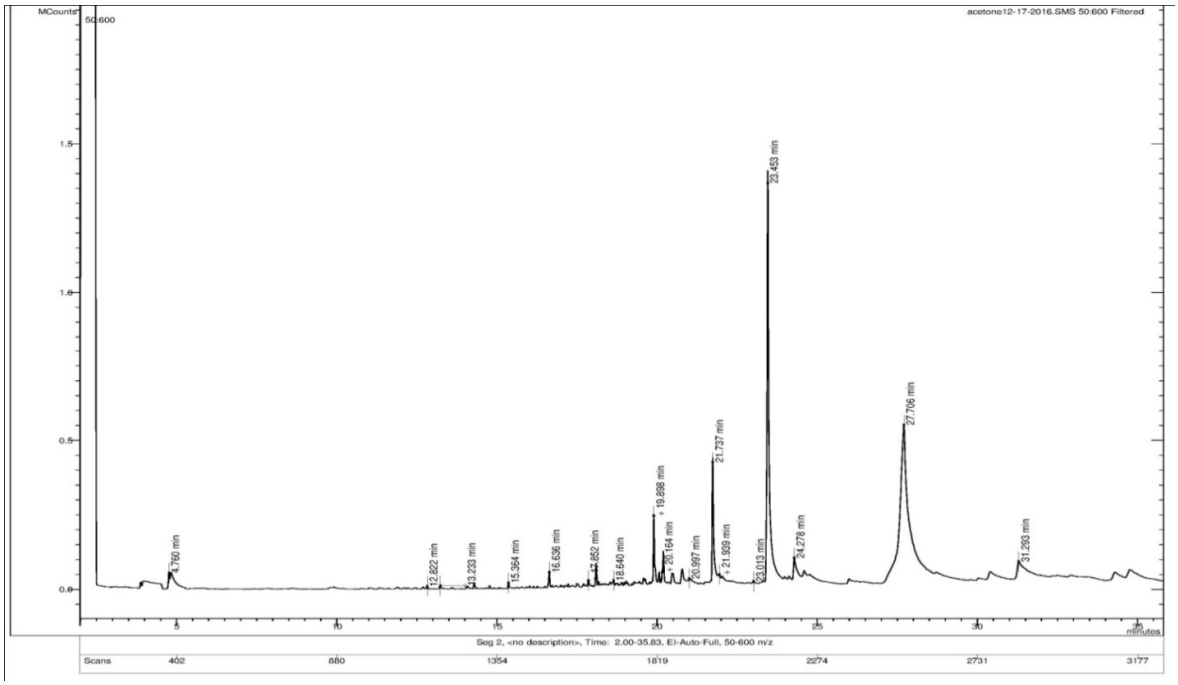

Figure 2 .Chromatogram of GCMS of acetone extract of the plant leaves of *Glycosmis pentaphylla*

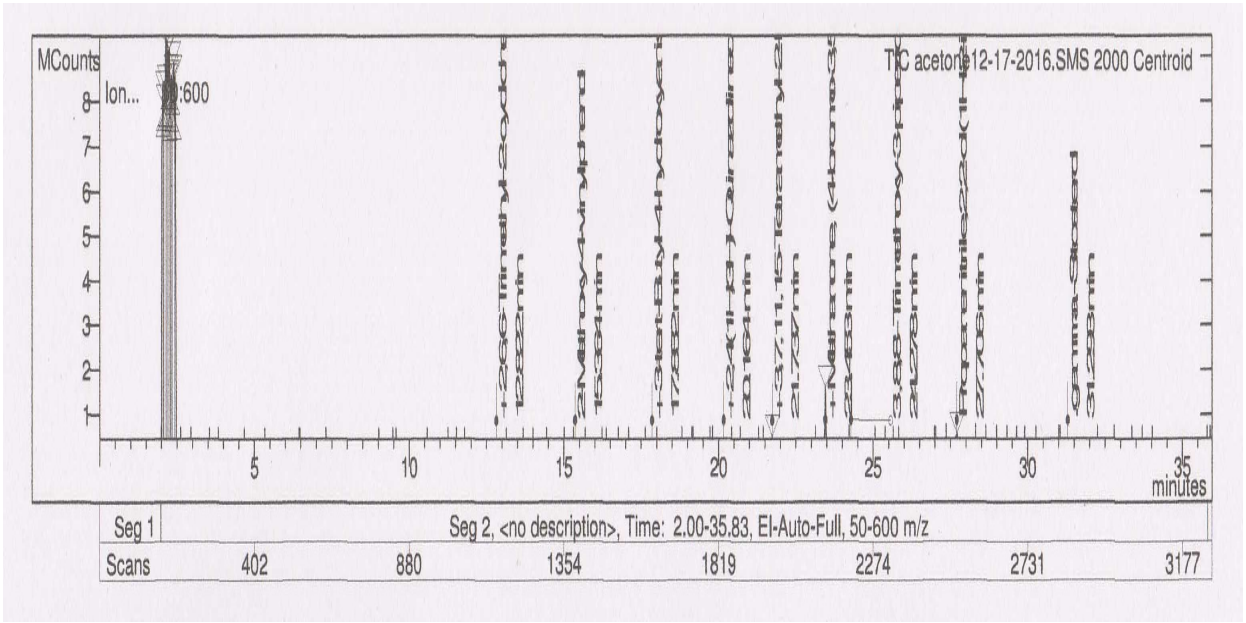

Figure 3. Graph showing GC MS analysis of acetone extract of the plant leaves of *Glycosmis pentaphylla*

Table 2.Compounds analysed in GC MS

SI no	RT	Peak name	Peak area	Peak %
1	11.037	Naphthalene	4685	0.185514
2	12.092	Phenol,2,4-bis(1,1-dimethyl ethyl)	4765	0.188682
3	12.809	Canophyllal	4589	0.181713
4	12.822	2,6,6-Trimethyl-2-cyclohexen-1	6734	0.266649
5	13.015	1-Heptadecene	5863	0.23216
6	13.203	Guaiol	5634	0.223092
7	13.233	1-Benzoylamino-5-piper	4985	0.197393
8	13.354	Rosifoliol	6834	0.270609
9	15.364	2-Methoxy-4-vinylphenol	12659	0.501264
10	16.636	Caryophyllene	8378	0.331748
11	17.852	3-tert-Butyl-4-hydroxyan	16026	0.634589
12	18.101	1H-Cycloprop[e]azulen-7	10409	0.41217
13	18.157	Caryophyllene oxide	2249	0.089055
14	18.640	4,4,5,8-Tetramethylchrom	4016	0.159023
15	19.153	n-Tetracosanol-1	4163	0.164844
16	19.309	2-isopropenyl-2,3-dihydro furo (3,2-g)chromen-7- 0ne	4089	0.161914
17	19.590	Cyclopenta(c)(1)benzopyran-4H(1H)-one,7-(dimethyl amino)-2,3 dihydro	5236	0.207332
18	19.924	9,12,15-Octadecatrienoico	5080	0.201155
19	20.064	Tricyclo[20.8.0.0(7,16)]heptane	5415	0.21442
20	20.164	2,4(1H,3H)-Quinazolinediol	41559	1.645631
21	20.459	Tricyclo[4.3.1.1(3,8)]undecanes	19166	0.758925
22	20.786	1,2-Benzene dicarboxylic acid bis(2-methylpropyl ester)	30768	1.218335
23	20.997	Ethyl iso-allocholate	1863	0.07377
24	21.270	1-methoxy benzene,44(2-hydroxybenzylidene amino)	1865	0.073849

25	21.683	Tetrapentacontan,1,54-di bromo	1960	0.077611
26	21.737	3,7,11,15-Tetramethyl-2-hexane-1-ol	106037	4.198797
27	21.939	Aspidospermidine-3-carboxylic acid, 2,3-didehydro-methyl ester	3247	0.128573
28	22.051	Oleic Acid	1128	0.044666
29	23.013	alpha.-Cedrene oxide	3643	0.144254
30	23.453	Methanone, (4-bromo-3,5 dimethyl phenol)	1271000	50.32838
31	24.023	Squalene	1985	0.078601
32	24.278	3',8,8'-Trimethoxy-3-pip	80743	3.197218
33	24.590	Hexatriacontane	1876	0.074285
34	26.565	Paverine	1868	0.073968
35	27.706	Propanenitrile,2,2-dimethyl azobis	820516	32.49036
36	31.293	gamma.-Sitosterol	14381	0.569451

DISCUSSION

In the present study, HPLC and GC MS analysis of the acetone extract of the plant *Glycosmis pentaphylla* showed the presence of many compounds The preliminary phytochemical investigations of acetone extract of *Glycosmis pentaphylla* indicates the presence of alkaloids, flavonoids phenolics, saponins, tannins ,steroids, carbohydrates etc.These secondary metabolites are reported to have many biological and therapeutic properties[12]. Similar results were obtained from the ethanol extract of plant leaves of *L.camara*.It contains lantadenes, triterpenes, steroids and alkaloids as major constituents [13]. *H. sauveolens* contains sabinene, diterpenes, 1, 8-cineole, limonene, suaveolol, triterpenoid, traces of hydrocyanic acid, sterol, etc [14].

The saponins content was also reported to be high in the phytochemical analysis of pericarp of *C. magna* [15]. Saponins are known for their anti-inflammatory antimicrobial and insecticidal properties [16]. Flavonoids have been reported to possess many useful properties, including estrogenic activity, insecticidal properties, anti-inflammatory and antimicrobial activity[17].

GC-MS chromatogram of acetone extract of *Glycosmis pentaphylla* revealed the pesence of 36 phyto compounds including Methanone, (4-bromo-3,5 dimethyl phenol), Propanenitrile,2,2-dimethyl-azobis,3,7,11,15-Tetramethyl-2-hexane-1-ol,gamma.-Sitosterol Caryophyllene etc.HPLC of acetone extract of *Glycosmis pentaphylla* revealed the pesence of 18 phyto compounds including 11-cis-Octadecenoic acid, Phospho-propylester, 1,4-Butanediamine (Putrescine), 1,2,3-Trioxybenzene etc . The detrimental effects of *Glycosmis pentaphylla* were probably associated with

the effects of these various phytochemical compounds present in it.Of these 18 phytocompounds, 72% of area occupied by 11-cis-Octadecenoic acid with 37.481 retention time.15% of area occupied by Phospho-propylester with 29.084 retention time.In 2008 study at the University of Alberta suggests that 11-cis-Octadecenoic acid feeding in rats over 16 weeks resulted in lowered total cholesterol, lowered LDL cholesterol and lower triglyceride levels.Putrescine, is a foul-smelling organic chemical compound. Putrescine is toxic in large doses. In rats it has a low acute oral toxicity of 2000 mg/kg body weight, with no-observed-adverse-effect level of 2000 ppm (180 mg/kg body weight/day) [18].

Phytocompounds such as oleic acid,glutamic acid etc have pesticidal property.Oleic acid is an unsaturated fatty acid used as herbicide, insecticide, fungicide. Monosodium Glutamate (MSG) or Ajinomoto is the sodium salt of glutamic amino acid, which is a non-essential amino acid.Farmers used msg as pesticides. Natural pyrethrin from the neem extract, *Azadirachta indica* were effective in controlling the cottonboll worm [19].Cyanogenic glycosides have been reported to yield prussic hydrocyanic acid which is a deadly poison. The negative result for cyanogenic glycosides confirms that the plant can be consumed by humans and it is also one good reason for its wide application as a stored product insecticide.

Many workers obtained similar findings with some other species of plants. Ethanolic/ Methanolic/ Chloroform/Hexane extracts obtained from different parts of the plant like root, leaves, aerial parts, stem, pods have been found to contain different chemical moieties: Alkaloids, glycosides, lignans, triterpenoid saponins, sterols, fatty acids, and coumaric acid derivatives [20].Acetone extract of different Plant leaves contain with alkaloids, phenol, steroids, protein, resins, steroids, tannins, terpenoids, cardiac glycosides, carbohydrates, saponins, glycosides, sugars, and catechol [21].

The chromatographic results showed the acetone extract of the selected plant contains various phytocompounds and the toxic effect may be assigned to the mixture or individual components. Present study supported with findings from some other workers.Preliminary phytochemical study of ethanol extract of Glycosmis pentaphylla showed the presence of alkaloids, gums, reducing sugars, tannins, flavonoids and saponins. Bioactivities like antimicrobial, membrane stabilization, anxiolytic activity and toxic effect of this plant is due to the presence of these phytochemicals [22].

The acetone extract of the *Glycosmis pentaphylla* showed that it had many volatile and nonvolatile compounds which have insecticidal activity.Presence of insecticidal compounds were confirmed by HPLC and GC MS.Since the plants are edible, medicinal and in view of the long history of human use, it stands a good chance of being the source of a new eco-friendly

bioinsecticide and grain protectant of natural origin. The test plants being non toxic would yield environmentally sound and economically feasible chemicals having no harmful effects on the non target organisms.

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