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Bioactive Compounds Investigation and TLC Studies of *Gymnema lactiferum* Leaves

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Abstract : *Gymnema lactiferum* leaves are consumed in Sri Lanka as remedies for preventing diabetes. This plant belongs to Asclepiadaceae family and this study was carried out to identify the bioactive compounds that synthesis this plant. Alkaloids, flavonoids, steroids, saponins, quinones, cardiac glycosides, phenol compounds protein and carbohydrates were compounds which detected in methanolic extract of *Gymnema lactiferum* leaves. Obtained results of thin layer chromatographic analysis revealed that totally 44 spots were detected in four extracts for different solvent system. 12 bands were observed for acetone extract meanwhile, 13,6 and 13 spots for ethyl acetate, hexane and methanol extracts respectively. Based on the results, *Gymnema lactiferum* leaves contained chemical compounds which have medicinal potentials.
Keywords : *Gymnema lactiferum*, Asclepiadaceae, Saponins, Quinones, Alkaloids.

Introduction

Nowadays, people are more aware of the herbal plants which reveal health benefits against many diseases. Currently, there are various types of research which have been carried out to identify and isolation of the plant secondary metabolites. Among them, some plants indicate some various phytochemicals that identify and use to synthesis drug[1]. Phytochemicals are compounds which are non-nutritive and produced by plants to protect themselves. These chemicals play and important role in health and also nutritionally. Secondary metabolites such as alkaloids, flavonoids, saponins, lycopene, antioxidants etc. facilitate the immune protection against some dangerous diseases. Alkaloids are the largest secondary compounds which contain one or more nitrogen atoms combined in part of a cyclic system[2]. *Gymnema lactiferum* is a climbing perennial shrub which is native to India and Sri Lanka. Vernacular name of *Gymnema lactiferum* is kurighghan. In Sanskrit, it is called ksirakakoli. Leaves of *Gymnema lactiferum* are consumed as a leafy vegetable as both raw and cooked forms[3].

Materials and Methodology

Collection and identification of plant

Fresh leaves of *Gymnema lactiferum* were collected from Kadawatha, Gampaha district between 8-10

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a.m. Then prepared herbarium specimen was identified and authenticated by National herbarium of Peradeniya, Sri Lanka.

Preparation of extraction

Thoroughly washed leaves with running tap water followed by distilled water and they were shade dried for 10 days and then it was ground into coarse powder and stored at 4 ^{0}C. 20g of powder was extracted with 80% methanol/water solvent system with use of Soxhlet apparatus for 6 hours. Extracted solution was evaporated using rotary evaporator at 40^{0}C. It was used to carried out tests.

Preliminary analysis of bioactive compounds

Determination of Saponins[4]

Frothing test

To 20 ml of distilled water, 100mg of powder was added and shaken for half an hour. Persistence froth revealed presence of saponins

Determination of Flavonoids[4]

Ferric chloride test

To 0.5ml of ferric chloride, 1ml of extract was added in a dry test tube and formation of woody brown colour was showed as positive result

Determination of Alkaloids[5]

Wagner's test

To 1ml of extract, 3 drops of Wagner's reagent was added in a dry test tube and formation of yellow colour precipitate was observed as positive result

Mayer's test

To 1 ml of extract, 3 drops of Mayer's reagent was added in a dry test tube and yellow colour precipitate revealed the positive result

Determination of Phenolic compounds[6]

Lead acetate test

To 1ml of extract, 3 drops of 1% lead acetate was added and formation of white colour precipitate revealed the positive result

Determination of Tannins[6]

Gelatine test

To 5 ml of distilled water, 3 ml of 10% of sodium chloride, 2 ml of 1% gelation solution and 50mg of extract were added and formation of white precipitate indicated the positive result

Ferric chloride test

To 0.5 ml of 5% $FeCl_3$, 1ml of extract was added and green colour precipitate revealed the positive result

Determination of Cardiac glycosides[7]

Keller-Killiani test

To 2ml of glacial acetic acid, 1ml of conc. H_2SO_4, 2 drops of $FeCl_3$ and 0.2g of coarse powder were added and formation brown colour ring at the interface revealed the positive for cardenolids

Determination of Quinones[7]

To 1ml of alcoholic KOH, 2ml of extract was added and formation of reddish blue colour showed the positive result

Determination of steroids[8]

To 2ml of acetic anhydride and 2ml of conc. H_2SO_4, 0.2g of powder was added and bluing colour revealed the positive result

Determination of Leucoanthocynidines[8]

To 2ml of extract, 1ml of conc. HCl was added followed by heating until it was boiled. Formation of reddish colour indicated the positive result

Determination of Phlobatannins[7]

To 2ml of extract, 2ml of 1% aqueous HCl was added followed by heating until it was boiled. Red colour precipitate indicated the positive result

Determination of Gum and Mucilage[8]

To 10 ml of distilled water and 2ml of absolute alcohol, 100 mg of dry powder was added and stirred constantly. Formation of white or cloudy precipitate indicated the positive result

Test for Protein[8]

Millon's test

To 2ml of methanolic extract, 6 drops of Millon's reagent was added and white colour precipitate indicated the positive results for this test

Determination of Carbohydrates[8]

Fehling's test

To 2ml of distilled water and 2ml of Fehling's A and B, 2ml of extract was added followed by heating until it was boiled. Formation of brick red colour precipitate indicated the positive result for carbohydrates

Bradford's test

To 2ml of methanol extract, 5 drops of Bradford's reagent was added. Formation of bluing colour precipitate indicated the positive result

Sample preparation for TLC

Pre-weighed, 3g of coarse powder was added into 100ml of each solvent including acetone, methanol, hexane, and ethyl acetate and then kept in a shaker for 72 hours. Extracted solution was then filtered and evaporated using a rotary evaporator

Thin layer chromatographic studies

For each extract, thin layer chromatography was carried out according to the one-dimensional ascending method. First, 6X7 cm of pre-coated silica plate was cut and bottom line was marked with use of a

soft pencil on 1cm above the plate. Extracts were dissolved in acetone and spots were applied on the plate using capillary tube and kept in chromatographic tanks which contained various solvent system including;(I) hexane: ethyl acetate (4:1), (II) hexane: ethyl acetate: methanol: water (5:3:1:1), (III) hexane: ethyl acetate: methanol (3:1:1) (IV) ethyl acetate: methanol (1:1) and then solvent front was marked and developed chromatogram was kept until it dry. Dried plates were visualized under daylight, 254nm and 365nm of ultraviolet light, and using an iodine chamber. For each separated compound, R_f value was calculated using the equation given below;

$$R_f = \frac{Distance\ moved\ by\ the\ solute/compound}{Distanced\ moved\ by\ the\ solvent} \quad (1)$$

Results and Discussion

Preliminary screening results of bioactive compounds were showed in table 01. Results revealed that *Gymnemalactiferum*leaves contained alkaloids, flavonoids, steroids, saponins, quinones, cardiac glycosides, phenol compounds, protein and carbohydrates. According to the previous study, tannins was present and flavonoids was absence in methanolic layer but presence in water extract and ethyl acetate extract[9]. Presence of the above bio active compounds have been reported different medicinal activities including alkaloids have antifungal properties, anti-parasitic activity[10], antibacterial[11] and flavonoids have anticancer[12], antioxidant[13], anti-allergic[14] anti-diabetes[15], anti-atherosclerotic activities[16]. Anti-cancer activity has been reported in saponins, polyphenols, steroids and quinones[17,18]. Carbohydrates and protein have nutritional value as energy sources as well as cell structural components[19].

Table 1: Phytochemicals screening results of *G. lactiferum*leaves

No.	Bioactive compound	Test/s	Methanol extract
1	Saponins	Frothing test	+
2	Flavonoids	Ferric chloride test	+
3	Alkaloids	Mayer's test Wagner's test	+ +
4	Phenol compounds	Lead acetate test	+
5	Tannins	Ferric chloride test Gelatin test	- -
6	Steroids		+
7	Cardiac glycosides	Keller-Killiani test	+
8	Quinones		+
9	Leucoanthocyanidines		-
10	Phlobatannins		-
11	Gum and mucilage		-
12	Protein	Millon's test	+
13	Carbohydrates	Fehling's test Bradford's test	+ +

(+) Presence; (-) Absence

According to the thin layer chromatographic study, 44 bands were detected and calculated retention factors for each separated band were tabulated in table 2 and figure 01 showed the developed four chromatograms. Twelve spots, 13,6 and 13 spots were detected on chromatograms of acetone, ethyl acetate, hexane and methanol extract respectively. Results revealed that most of the bands were detected in polar solvents and solvent system I was the best solvent system among four systems that 23 bands were separated for acetone, ethyl acetate, hexane and methanol extracts. 9, 8 and 4 bands were separated in solvent system II, solvent system III and solvent system IV respectively.

Table 2: Results of TLC for *Gymnema lactiferum* leaves

Extract	**Solvent system I**		**Solvent system II**		**Solvent system III**		**Solvent system IV**	
	No. of spots	$R_f$value	No. of spots	$R_f$value	No. of spots	$R_f$value	No. of spots	$R_f$value
Acetone	7	0.18 0.33 0.43 0.52 0.64 0.75 0.81	2	0.10 0.18	2	0.81 0.90	1	0.46
Ethyl acetate	6	0.14 0.33 0.43 0.52 0.62 0.81	3	0.10 0.18 0.90	3	0.73 0.90 0.97	1	0.63
Hexane	4	0.33 0.66 0.79 0.91	1	0.96	0	0.00	1	0.75
Methanol	6	0.16 0.33 0.37 0.47 0.62 0.77	3	0.09 0.72 0.87	3	0.78 0.90 0.97	1	0.57

Solvent system I **Solvent system II** **Solvent system III** **Solvent system IV**

Figure 1: Results of thin layer chromatograms

Conclusion

In conclusion, *Gymnema lactiferum* leaves contained pharmaceutically and nutritionally important compounds including alkaloids, flavonoids, steroids, saponins, quinones, cardiac glycosides, phenol compounds due to their medicinal activities as well as the presence of carbohydrates and proteins.

References

1. Sofowora A. Research on Medicinal Plants and Traditional Medicine in Africa," The J Alter Com Med., 1996, 2; 365-370.
2. Harbone JB., Phytochemical methods: A guide to modern technique of plant analysis, Champman and Hall, London, 1983,215.
3. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Influence of Boiling, Steaming and Frying of Selected Leafy Vegetables on the In Vitro Anti-inflammation Associated Biological Activities. Plants., 2018, 7; 22—30.
4. Singh R, Kumar A, Giri DD, Bhuvaneshwari K, Pandey KD. Gas chromatography- Mass Spectrometry analysis and phytochemical screening of methanolic fruit extract of Momordica charantina. J Res Adv Agri., 2012, 1; 122-127.
5. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. Int J Advan Res Chem Sci., 2015, 4; 25-32.
6. Cynthia AI, Nivethini L, Victoria TD. Phytochemical screening and bioactivity of Momordica charantina L. J Chem Pharm Res., 2015, 7; 970-975.
7. Trease GE, Evans WC., Pharmacognosy: Nigerian, Trldercan Macmillian publisher, New York, 1989, 55.
8. Caron C, Hoizey MJ, Men-Olivier LL, Massiot G, Zeches M, Choisy C, Magrex EL, Verpoorte R. Antimicrobial and antifungal activities of quasi-dimeric and related alkaloids. Plan Med., 1998, 5; 409–412.
9. Kluza J, Mazinghien R, Degardin K, Lansiaux A, Bailly C. Induction of apoptosis by the plant alkaloid sampangine in human HL-60 leukemia cells is mediated by reactive oxygen species. Euro J Pharm., 2005, 525; 32–40.
10. Ferreyra FML, Rius S, Emiliani J, Pourcel L, Feller A, Morohashi K, Casati P, Grotewold E. Cloning and characterization of a UV-B-inducible maize flavonol synthase. Plan J., 2010, 62; 77–91.
11. Mierziak J, Kostyn K, Kulma A. Flavonoids as Important Molecules of Plant Interactions with the Environment. Mole., 2014, 19; 16240-16265.
12. Kritas SK, Saggini A, Varvara G, Murmura G, Caraffa A, Antinolfi P, Toniato E, Pantalone A, Neri G, Frydas S. Luteolin inhibits mast cell-mediated allergic inflammation. J Biol. RegulHomeoAgen., 2013, 27; 955–959.
13. Gaur R, Yadav KS, Verma RK, Yadav NP, Bhakuni RS. In vivo anti-diabetic activity of derivatives of isoliquiritigenin and liquiritigenin. Phytomed., 2014, 21; 415–422.
14. Salvamani S, Gunasekaran B, Shaharuddin NA. Antiartherosclerotic effects of plant flavonoids. BioMed Res., 2014, 2014,1-11.
15. Patel RP, Boersma BJ, Crawford JH, Hogg N, Kirk M, Kalyanaraman B, Parks DA, Barnes S, Darley-Usmar. Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. Free Rad Bio Med, 2011, 31; 1570-1581.
16. Tsao R. Chemistry and Biochemistry of Dietary Polyphenols. Nat., 2012, 2; 1231-1246.
17. Sultan A, Raza AR. Steroids: A Diverse Class of Secondary Metabolites. Med Chem., 2015, 5; 310-317.
18. Eyong KO, Kuete V, Efferth T., Medicinal plant research in Africa, Elsevier, New York, 2013,395.
19. Inaghe OM, Malomo SO, Adebayo JO. Proximate composition and phytochemical constituents of leaves of some Acalypha species. Pak J Nutri., 2013, 8; 256-260.

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