J. Phytol. Res. **31** (1-2) : 17-22, 2018 **ISSN 0970-5767**

PHYTOCHEMICAL ANALYSIS OF LEAF AND BARK EXTRACTS OF *M. HEXANDRA (ROXB.)*- A VALUABLE MEDICINAL PLANT

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Phytoconstituents from leaf and bark extracts of *M. hexandra* have been analyzed quantitatively and qualitatively by respective standard procedures. Plant contain rich contents of carbohydrate and protein which was estimated by phenol- sulphuric acid method and Lowry's method respectively. Total carbohydrate contents were found 39% and 32.35% and total starch contents were found 44.95% and 38.1% of dry mass in leaf and bark respectively. Total protein content was estimated 5.03% in leaf and 32.9% in bark. Total lipid content was found low 0.05 % for both extracts. Total phenol content was estimated via folin ciocalteau method and it was found 0.52% in leaf and 1.46% of dry mass in bark. Soxhlet extraction procedure was used to prepare methanol, petroleum ether and chloroform extracts for estimation of presence of various secondary metabolites such as alkaloids, flavonoids and sterols and their presence were identified via Thin layer chromatography. Various standard analytical tests were also performed for detection of both primary and secondary metabolites in leaf and bark extracts of *M. hexandra*. The findings of the study provided evidence that the stem bark and leaf of the plant possessed bio active compounds. It justifies their use in the traditional medicines for the treatment of different diseases.

Keywords: *M. hexandra*, Phytochemistry, Primary and Secondary metabolites.

Introduction

Mimusops hexandra (Roxb.) is a medicinal and socio-economic plant species of tropical deciduous forests of western and central India, belongs to family Sapotaceae[1]. Bark, fresh fruits and seeds of *M. hexandra* have high economic value due to its nutritional and medicinal applications[2]. Plant used as a significant source of livelihood and nutritional support for tribal people. Extracts and metabolites of this plant possess huge biological importance[3]. Stem bark possess medicinal properties such as antiulcer, anti-inflammatory antidiabetic and antibacterial activity[4,5,6]. Decoctions of stem bark widely used to cure dysentery, diarrhea, body ache, fever and hallucinations[7,8,9], whereas the infusion used as galactagauge[10]. Leaves of *M. hexandra* are traditionally used as anti-inflammatory, anti-urolithiatic, anti-pyretic, anti-microbial, analgesic and diuretic activities. Leaf extract used in treatment of asthma[11]. Madhak et al[12] observed the presence of sterols volatile oil, tannin via phytochemical analysis of alcoholic extracts of leaves. Misra and Mitra[13] observed the presence of cinnamic acid, hentriacontane, taraxerol and quercitol in aqueous and

alcoholic extracts of leaves. Triterpenoid saponin and B-sitosterol have been isolated from the stem bark of *M. hexandra* and their structures elucidated on the basis of chemical and spectral evidence[14]. However, only a small proportion has been investigated phytochemically. Present study aims to analyze phytoconstituents of *M. hexandra* via standard procedures which may generate new information for drug discovery from this valuable medicinal plant.

Material & Methods

The air- dried and grinded powder of leaves and bark used for qualitative analysis. Biochemical tests specific for particular metabolite have been performed and the reaction responses were noted to ensure the presence of particular compound. Carbohydrates were tested by Fehling's, Benedict's, Molisch's and Iodine test, whereas for proteins Biurate, Ninhydrin and Millon' s test were used. Lipids were tested by Acrolein and Sudan IV test[15,16]. Plant sample for quantitative estimation of carbohydrates were prepared by method of Loomis and Shull[17]. Starch samples were prepared from the residue of total extracted sugar samples by method of McCready et al[18]. Phenol-sulphuric acid method was used for estimation of net content of carbohydrates[19]. 5% phenol and concentrated sulphuric acid was used for separation of total soluble sugars. Protein content was estimated by method of Lowry et al[20]. Samples of leaf and bark was prepared via method of Osborne by using cold trichloric acid[21]. Solution of 2% Na_2Co_3 and 0.5% $CuSO_4$ was also used with addition of diluted folin ciocalteau reagent for extraction of proteins.

Further, the optical densities of used standard sugar (glucose) and protein (bovine serum albumin) with their respective samples was measured at 490 and 750 nm wavelength using spectrophotometer where distilled water was used as blank. Regression curve was prepared between the known concentration of glucose and BSA & their respective absorbance which followed and Beer- Lambert law[22]. Net contents were calculated from regression curve by using equation of standard line. Similarly, for the estimation of total phenolic contents, Thorpe and Bray's[23] protocol was used by using ethanolic solution of gallic acid as standard. Folin ciocalteau reagent and Na_2Co_3 solution was added for extraction of phenolic contents[24]. The optical density of gallic acid and plant samples were observed at 750 nm and net phenolic contents were calculated as previous. Solution of chloroform and methanol (2:1, v/v) was used for estimation of net lipid content[25]. The lipids were separated with chloroform and collected in the pre-weight glass vials then weighed. The procedure repeated for three times and mean values were calculated.

Various biochemical test used in estimation of presence of secondary metabolites were also performed. Mayer's, Wagner's, Dragendorff's and Hager's test was used to identify presence of alkaloids, Shinoda test and Alkaline reagent test was used for flavonoids, Liebermann-Burchard's and Salkowaski test was used for sterols. Ferric chloride test was used for phenols[26]. Qualitative estimation of alkaloids was done via gravimetric method in which methanolic extracts of leaf and bark were prepared by soxhlet extraction and further extracted by chloroform. The free alkaloids were separated by ammonia[27]. The extracts were further analyzed by Thin Layer Chromatography. Flavonoids have been separated from powdered samples of leaf and bark with petroleum ether and 80%

methanol via soxhlet extraction at 45-60 ºC. Then again fractioned by sequential extraction with petroleum ether, ethyl ether and ethyl acetate separately. Ethyl ether and ethyl acetate fractions were used for estimation of flavonoids[28]. Identification of sterols have been done by using petroleum ether for separation of fats from dried plant samples. The dried preparation was again extracted with benzene and further proceeds for TLC[29].

Thin Layer Chromatography

Thin silica containing glass plates were used for chromatographic separation. The extracted samples were used for chromatographic separation and co-chromatographed with authentic alkaloids such as colchicine, flavonoid such as Kaempferol and sterol such as B-sitosterol in the chromatographic chamber saturated with solvent mixture of methanol and conc. ammonium hydroxide at ratio of 200:3, n-butenol, acetic acid and water at ratio of 4:1:5 and hexane and acetone at the ratio of 8:2 for alkaloids, flavonoids and sterols respectively[30]. The spots were identified coinciding with the colchicine, kaempferol and B- sitosterol marker. Ammonia fumes was used to darken the spots. The developed plated were air The dried and visualized under ultra violet light. The retention factor (R_f Value) of each spot were calculated.

Results and Discussion

The biochemical tests show positive responses in all reactions. Observed responses such as formed precipitate and change in color of the solution indicates the presence of particular primary and secondary metabolite. Net contents of carbohydrate, starch, protein and phenols analyzed by quantitative estimation has shown in table 1.

Presence of various secondary metabolites such as alkaloids, flavonoids and sterols were also identified by Thin layer chromatography using respective standards. They appear as a single spot and have the same color and retention factor (R_f) value nearly equivalent to their standards on the TLC plate. The R_f values of these compounds in the different solvent systems were calculated as in table 2.

Infusions and decoctions of *M. hexandra* have been used to cure most common to severe diseases by local inhabitants since long time period. Bioactive compounds such as taraxerol, quercitol and B-sitosterol etc. have been identified and isolated from different parts of the plant previously.

Table 1: Net content of various phytoconstituents found in leaf and bark extracts of *M. hexandra*.

Phytoconstituent	Plant part	Net content found(mg/ml)	% Dry weight
Carbohydrates	Leaf	0.780	39%
	Bark	0.647	32.35%
Starch	Leaf	0.899	44.95%
	Bark	0.762	38.1%
Protein	Leaf	0.151	5.03%
	Bark	0.988	32.9%
Lipid	Leaf	0.05	0.05%
	Bark	0.05	0.05%
Phenol	Leaf	0.104	0.52%
	Bark	0.292	1.46%

Table 2 Estimation of the presence of various secondary metabolites in leaf and bark extracts of *M. hexandra* using Thin layer chromatography.

Phytoconstituent	Solvent system	Plant part	R_f value
Alkaloids	Methanol and conc. ammonium hydroxide (200:3)	Leaf	0.50
		Bark	0.61
Flavonoids	n-Butenol, acetic acid and water (4:1:5)	Leaf	0.30
		Bark	0.50
Sterols	Hexane and acetone (8:2)	Leaf	0.78
		Bark	0.80

In this study secondary metabolites such as alkaloids, flavonoids, phenols and sterols have been identified in leaf and bark extracts of *M. hexandra*. This work may aid in further qualitative and quantitative characterization of secondary metabolites which may be help in discovery of novel drugs.

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