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MORPHOLOGICAL, PHYTOCHEMICAL AND PHYSICO-CHEMICAL PROFILING ON LEAF EXTRACT OF FOUR MENISPERMACEAE SPECIES FROM KERALA

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ABSTRACT

The Menispermaceae family of flowering plants comprises of about 71 genera and 450 species. Menispermaceae are mostly climbing plants and the majority of species can be found in countries with a tropical climate. The family contains a number of plants with scientifically recognized important pharmacological activities. The present work aimed at leaf morphology, preliminary phytochemical screening of four medicinally important species of the family Menispermaceae using two solvent systems (chloroform and ethanol) and also to evaluate physico-chemical characterisation of these extracts. The selected species include *Anamirta cocculus* (L) Wight & Arn., *Cissampelos pareira* L. Var. *hirsuta* (Buch.-Ham. ex DC.) Forman, *Pachygone ovata* (Poiret) J. D. Hook. & Thompson and *Diploclisia glaucescens* (BI.) Diels. The powdered leaves were extracted with two solvents with Soxhlet apparatus and the extracts were subjected to preliminary phytochemical screening by standard laboratory tests. Results showed that the chloroform leaf extracts show more positive result than that of ethanolic extracts. The extractive value (yield percentage) in two solvent system showed that ethanolic extract has the maximum yield percentage than that of chloroform extract. The study showed that Chloroform and Ethanol leaf extracts of selected plants contain medicinally improved bioactive compounds and also justifies the use of these plants as traditional medicines and for developing pharmaceutical drugs.

Keywords: Morhological, Phytochemical, Physico-chemical, medicinal plants, *Anamirta cocculus* , *Cissampelos pareira* var. *hirsuta, Pachygone ovata*

INTRODUCTION

The Menispermaceae comprises of 71 genera with 450 species **[1]**. In kerala family comprises of 11 genera and 19 species **[2]**. Menispermaceae are mostly climbling plants and majority of species can be found in countries with tropical climate. The main feature historically used to define the family is the curved seed found in many of the genera, hence the common name moonseed family. Members of the family are mostly lianas, sometimes small trees or shrubs and occasionally perennial herbs. The leaves are alternate, petiolate, sometimes peltate and exstipulate. The lamina is simple, entire or lobed, palmately veined **[3, 4]**. The family contains a number of plants with scientifically recognized important pharmacological activities.

The selected species include *Anamirta cocculus* (L) Wight & Arn., *Cissampelos pareira* L. Var. *hirsuta* (Buch.-Ham. ex DC.) Forman, *Pachygone ovata* (Poiret) J. D. Hook. & Thompson and *Diploclisia glaucescens* (BI.) Diels in the family Menispermaceae

Anamirta cocculus is a large, dioecious climbing plant. The stems twine into other plants for support. In South-East Asia the fruit of *Anamirta cocculus* is used mainly as a fish poison and as an insecticide. *Anamirta cocculus* contain a powerful poison picrotoxin. The poisoning affects the CNS of vertebrates causes vomiting, purging, profuse sweating, dimness of vision, unconsciousness, intoxication and clonic convulsions **[5]**.

Cissampelos pareira is sub- erect or small climbing twinner with velvety branches. A decoction is given in colic and dysentery, cholera and bronchitis **[6]**. The root acts as an antiseptic of the bladder and is used in chronic inflammation of urinary passage **[7]**. Traditonally, *C.pareira* is reported for its blood purifier and anti inflammatory properties in India **[8]**.

Pachygone ovata is a climbing shrub with pubescent branchlets. *Pachygone ovata* has been an unexplored and trivialized plant **[9]** that belongs to the Menispermaceae family. The ancient folks considered this whole plant to be medicinally important, since the dried fruit was used as fish poison and vermicide. The leaves were used to reduce body temperature and improve fertility and it also possesses various pharmacological actions including Analgesic, CNS stimulant and to cure hypothermia & leucorrhea **[10]**.

Diploclisia glaucescens is a Woody climber. It contains many bioactive compounds that justify the use of this plant as traditional medicines and for developing pharmaceutical drugs. A decoction of leaves is taken in two cups doses two times in a day for rheumatism **[11]** (*Diploclisia glaucescens*, Menispermaceae).

MATERIALS AND METHODS

Collection of the plant material

Whole plants of *Anamirta cocculus, Cissampelos pareira* var *hirsuta, Pachygone ovata, Diploclisia glaucescens* were collected from different parts of Kerala.

Morphological analysis

Macroscopic analysis of the selected plant leaf was carried out according to the method of Evans **[12]**. Parameters such as Leaf shape, Leaf apex, Leaf base, Margin, Venation, Leaf arrangement, Hairs, Domatia, Petiole, and Stipule were studied and recorded.

Preparation of plant leaf extract

The collected plant parts were cleaned, shade dried and powdered by a mechanical grinder. 10 g of the sample was extracted in a Soxhlet apparatus using 150 ml of chloroform and ethanol as the solvent, and the Soxhlet was run overnight until the sample loaded became colorless. After the Soxhlet extraction is over, extracts were put inside rotor evaporator until it gave the solidified residues. The sample extracts were collected, weighed and stored in a freezer.

Preliminary Phytochemical Screening

The chloroform and ethanol leaf extracts of *Anamirta cocculus, Cissampelos pareira* var *hirsute, Pachygone ovata, Diploclisia glaucescens* were subjected to qualitative test to identify various constituents. Aliquot portion of the crude leaf extract residue were weighed and used for phytochemical screening. Phytochemical screening was performed using standard procedure. Carbohydrate, Protein Aminoacid, Tannin and phenolic compound, terpenoid, Steroid, Glycosides, Quinone, Anthraquinone, Saponin, Alkaloid, Coumarin, Flavanoid and Resin were qualitatively analysed **[13-16]**.

Tests for carbohydrates

Fehling's test: Aqueous dispersion (2 mL) of extract was treated with 2 mL of Fehling's solution and heated on boiling water bath for 2 minutes, and then cooled. Appearance of reddish orange precipitate indicates the presence of carbohydrate.

Molisch's test: To 2 mL aqueous dispersion of extract, added few drops of alpha naphthol solution in alcohol, shaken and added concentrated sulphuric acid through the side of the test tube. Appearance of reddish violet ring at the junction indicates the presence of carbohydrate.

Iodine test: Mixed 3 mL aqueous dispersion of extract and a few drops of dilute iodine solution. Appearance of blue or violet colour indicates the presence of non reducing polysaccharide (starch).

Test for proteins

Biuret test: To 3 mL test solution, added 4% sodium hydroxide solution and few drops of 1 % copper sulphate solution.

Appearance of violet or purple colour indicates the presence of proteins.

Millon's test: Mixed 3 mL test solution with 5 ml Millon's reagent. Appearance of red precipitate or solution indicates the presence of proteins.

Test for Amino acids

Ninhydrin reagent: To 2 mL of aqueous solution of alcoholic extract added 1 drop of N inhydrin reagent (1% w/v). Bluish violet colour developed on addition of ninhydrin reagent indicated the presence of amino acid.

Test for tannins and phenolic compounds

To 1 mL of gelatin solution, added 1ml of aqueous solution of extract. Appearance of precipitation indicates the presence of tannins and phenolic compounds. (b) To 2 mL of aqueous solution of alcoholic extract added 0.5 ml of ferric chloride solution. Appearance of blue colour indicates the presence of tannins and phenolic compounds.

Test for terpenoid

3ml of extract was taken with 3ml of chloroform followed by few drops of concentrated sulphuric acid and shaken well. Appearance of reddish brown precipitate indicates the presence of terpenoids.

Test for steroids Salkowski reaction: To 2 mL of extract, added 2 mL chloroform and 2 mL concentrated sulphuric acid and shaken well. Appearance of red colour in chloroform layer and greenish yellow fluorescences in acid layer indicates the presence of steroids.

Liebermann's reaction: Mixed 3 mL extract with 3 mL acetic anhydride. Heated and cooled. Added a few drops of concentrated sulphuric acid. Appearance of blue colour indicated the presence of steroids.

Test for Glycosides

I ml of extract, glacial acetic acid , one drop of 5% ferric chloride and concentrated sulphuric acid was added along the sides of the test tube. Appearance of reddish brown colour at the junction of two liquid layer indicates the presence of glycosides.

Test for Quinone

Concentrated sulphuric acid (1ml) was added to 1 ml of plant extract. The formation of red colour indicates the presence of quinone.

Test for Anthraquinones

Few drops of 2% HCl were added to 0.5 ml of extract. Appearance of the red colour indicates the presence of anthraquinones.

Test for Saponins

Foam test: 1ml of extract mix with 20 ml distilled water in 100ml measuring cylinder for 15 minutes. Appearance of 1cm form indicates the presence of saponins.

Test for alkaloids.

A little of the dry extract was dissolved in few drops of dilute HCl acid and filtered.

The filtrate was tested for the presence of alkaloids.

Mayer's test: Mixed 1 ml of the filtrate with 2-3 drops of Mayer's reagent in a watch glass. Appearance of a pale yellow colour indicates the presence of alkaloids.

Hager's test: One ml of the filtrate was mixed with equal volume of Hager's reagent. Appearance of orange brown precipitate indicates the presence of alkaloids.

Test for Coumarins

10% of NaOH (1ml) was added to 1 ml of the plant extract. The formation of intense yellow colour indicates the presence of coumarins.

Test for Flavanoids:

5ml of dilute ammonia was added to a portion of aqueous filtrate of extract. Then 1ml of concentrated sulphuric acid is added. The formation of yellow colour indicates the presence of flavanoids.

Test for Resin

5ml of CuSO4 was added to 5ml extract. The resultant solution was shaken vigorously. The formation of green colour precipitation indicates the presence of resins.

Physico- Chemical Characterisation

Physico- Chemical Characterisation of selected plant species was evaluated. Parameters such as Moisture content, Total ash content, Extractive value, pH, Physical appearance of the extract and Consistency **[17]** were evaluated.

Determination of loss on drying (Moisture content)

Two grams of crude powder was taken in an evaporating dish and then dried in an oven at 105°C till constant weight was obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

Determination of total ash

Two grams of dry powder was taken in a silica crucible and heated gradually increasing the heat to 500°C until it was white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without delay.

Then the percentage of total ash was calculated with reference to the air-dried drug.

Total ash value (%) = Weight of ash/Weight. of drug X 100

Determination of Extractive value

The extracts were concentrated by using rotor evaporator until it gave the solidified residues. The sample extracts were collected, weighed. The extractive value in percentage was calculated by using formula.

Extractive value (%) = Weight of dried extract /Weight of plant material X 100

RESULT AND DISCUSSION

The four species were collected in different part of Kerala. They were examined for their proper identification. Collection

locality and their vernacular names were given in **Table 1 and Figure 1 (A, B, C and D)**.

Macroscopic characters of leaves

Morphological characters of selected plants were examined and noted carefully. Parameters such as Leaf shape, Leaf apex, Leaf base, Margin, Venation, Leaf arrangement, Hairs, Domatia, Petiole, and Stipule were noted **(Table 2** and **Figure 2)**. The results showed that *Anamirta cocculus* has ovate leaf with acuminate apex and cordate to truncate base. Leaf arrangement is alternate spiral with entire margin and palmate venation. Domatia present as hairy patches in the axils of main and secondary vein. Long Petiole swollen at both ends (Geniculate base). Whereas in case of *Cissampelos pareira* var *hirsuta* leaf is peltate with rounded apex and cordate base **[18]**. Leaf arrangement is Alternate spiral with ciliate margin and finely palmate venation. Long hairy petiole inserted with in basal margin.

The results also showed that *Pachygone ovata* has ovate –lanceolate leaf with mucronate apex and obtuse rounded base. Alternate distichous leaf arrangement with entire margin and cross venulate venation. Haired petiole and pulvinus present on petiole and this is quite common in the Menispermaceae family as also seen in Cocculus hirsutus **[19]**. Whereas in case of Diploclisia *glaucescens* the leaf is round to kidney shape with obtuse apex and truncate base. Alternate spiral leaf arrangement with entire margin and pinnately veined. Long and thin stalked petiole usually longer than lamina.

Macroscopic features of Pachygone ovata highlighted ovate lanceolate leaves with an entire margin showing reticulate veination, microscopic analyses indicated anomocytic type of stomata on the abaxial surface, presence of starch grains in the parenchyma layer and unicellular and multicellular trichomes on the midrib **[20]**.

Phytochemical screening

Phytochemical investigation of selected plants in two different solvent systems revealed that chloroform extract shows more secondary metabolites than ethanolic extracts **(Table 3)**. The results also revealed the presence of steroids, alkaloids, coumarins, flavanoids, resin in *Anamirta cocculus* leaf. The seed extracts of *A. cocculus* contained various pharmaceutically active substances *such as* aldehydes, alkaloids, phenolic compounds, flavonoids, saponins, carbohydrates, proteins, lipids, glycosides, phytosterols, volatile oils, gums and mucilage. *Anamirta cocculus* seed can serves potential source of pharmaceutically important bioactive compounds which could be useful in controlling the growth of various pathogenic bacteria as it posses various

secondary metabolites having the potential for developing pharmaceutical drugs **[21]**.

Secondary metabolites such as carbohydrate, protein, steroids, glycosides, qunoine, anthraqunoine, alkaloid, coumarin, resin were observed in *Cissampelos pareira* var *hirsuta* Morphological investigation of *Cissampelos pareira* revealed colour of leaves as greenish on outer side and grayish underneat, Cordate shape, apex of the leaf as obtuse or Emarginate. The leaf showed entire margin, unequal bases, finely palmate venation and peteolated **[22]**.

Phytochemical screening revealed that the *Cissampelos pareira* root extract contains terpenoids, alkaloids, tannins, amino acid proteins, and carbohydrates. Alkaloids and essential oil were also detected in TLC of the *Cissampelos pareira* root extract **[23]**. Different chemical compounds such as triterpene, flavonoids, glycosides, alkaloids, and carbohydrates were detected in the *Cissampelos pareira* plant, which could make the plant useful for treating different ailments .Traditionally and experimentally, it has been found that the *Cissampelos pareira* root is a potential herbal medicinal agent **[24]**.

The results also revealed the presence of carbohydrate, protein, aminoacid, tannin and phenolic compound, terpenoid, steroid, quinine, alkaloid, coumarin, resin in *Pachygone ovata* and presence of carbohydrate, protein, tannin and phenolic compound, terpenoid, steroid, quinine, anthraquinone, saponin, alkaloid, coumarin in *Diploclisia glaucescens.*

Physico-chemical characterisation

Various physico-chemical parameters of leaf powder and extract were quantitatively determined and were tabulated in the **Table 4**. Parameters such as moisture content, total ash content , physical appearance of the extract and percentage yield were examined. The physico-chemical studies are done to ensure uniformity in quality of formulations. It is a parameter in quality control therefore avoiding adulteration of herbal drugs **[25]**. The physico-chemical and phytochemical evaluation can be useful in identifying the authenticity, all of these together serve as a standard data for distinguishing *P. ovata* from other similar plants.

Moisture content is more in *Anamirta cocculus* and less in *Pachygone ovata*. Moisture content could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application. The results obtained from the pharmacognostical and phytochemical investigation of the roots of *Pachygone ovata* showed Moisture content as 6.78 and Total ash as 6.5. The parameters can be

used for the further investigation of the *Cissampelos pareira* Linn. var. *hirsuta* roots **[26]**. Physico-chemical parameters of *Pachygone ovata* indicated low amounts of moisture (3.45) and low amounts of acid insoluble ash (2.01) which helped in concluding the nature of the compounds and secondary metabolites present in the plant material, confirming the presence of flavonoids, triterpenoids, glycosides and alkaloids **[27]** .

Ash values are used to determine purity of crude drug. It indicates presence of earthly material, organic and inorganic compound and various impurities like carbonate, oxalate and silicate. The resulted revealed that the total ash value is more in *Cissampelos* and less in *Pachygone ovata*. The total ash, acid insoluble ash and water soluble ash were found to be 0.78%, 0.41% and 0.04% respectively. The alcohol and water soluble extractive values are 6.78% and 3.65% respectively **[28]**. The results also showed that chloroformic extract showed more percentage yield than the ethanolic extract in all selected plants. The extractive values provide a key role to evaluate the chemical constituents present in the crude drug and also helps in the estimation of specific constituents soluble in a particular solvent **[29, 30]**.

the seeds of A. cocculus possess various secondary metabolites having the potential for developing pharmaceutical drugs, especially antimicrobial ones. The seeds of *A. cocculus* possess various secondary metabolites having the potential for developing pharmaceutical drugs, especially antimicrobial ones. *A. cocculus* seeds can serve as a potential source of pharmaceutically important bioactive compounds which could be useful in controlling the growth of various pathogenic bacteria.

Leaves of *Diploclisia glaucescens* are most commonly used to cure various human ailments. Studies showed that leaf ethanolic extracts were active against *E.coli, Pseudomonas aeruginosa, Salmonella paratyphi.* Phytochemical studies revealed the presence of tannins, alkaloids and, proteins and other biocompounds. The physico-chemical and phytochemical evaluation helps in identifying the authenticity to serve as a standard data for distinguishing the plant species from other similar plants **[27]**.

Table 1: Vernacular name and Locality of selected plants

Taxon	Vernacular name English	Vernacular name Malayalam	Locality
Anamirta cocculus	Fish berry, poison berry, levant berries	Kollakaya, nanchuvalli, pettumarunnu	Bonacaud (8° 40.6045' N 77° 6.0863' E)
Cissampelos pareira var.*hirsuta*	Velvet leaf, false pareira brava	Malathaanti, paatathali	Wayanad 11° 47.2021' N 75° 59.2966 E
Pachygone ovata	Broom creeper	Katukodyvally, Kadukkodi	Palode (8° 43.466' N 77° 1.4869' E)
Diploclisia glaucescens	Glaucous Diploclisia	Battavalli, vattavalli, vattolli	Idukki (10°18.00'N 77°10.30'E)

A- *Anamirta cocculus*

B- *Cissampelos pareira* var.*hirsuta*

C -*Pachygone ovata*

D- Diploclisia glaucescens

Figure 1: Selected Plants

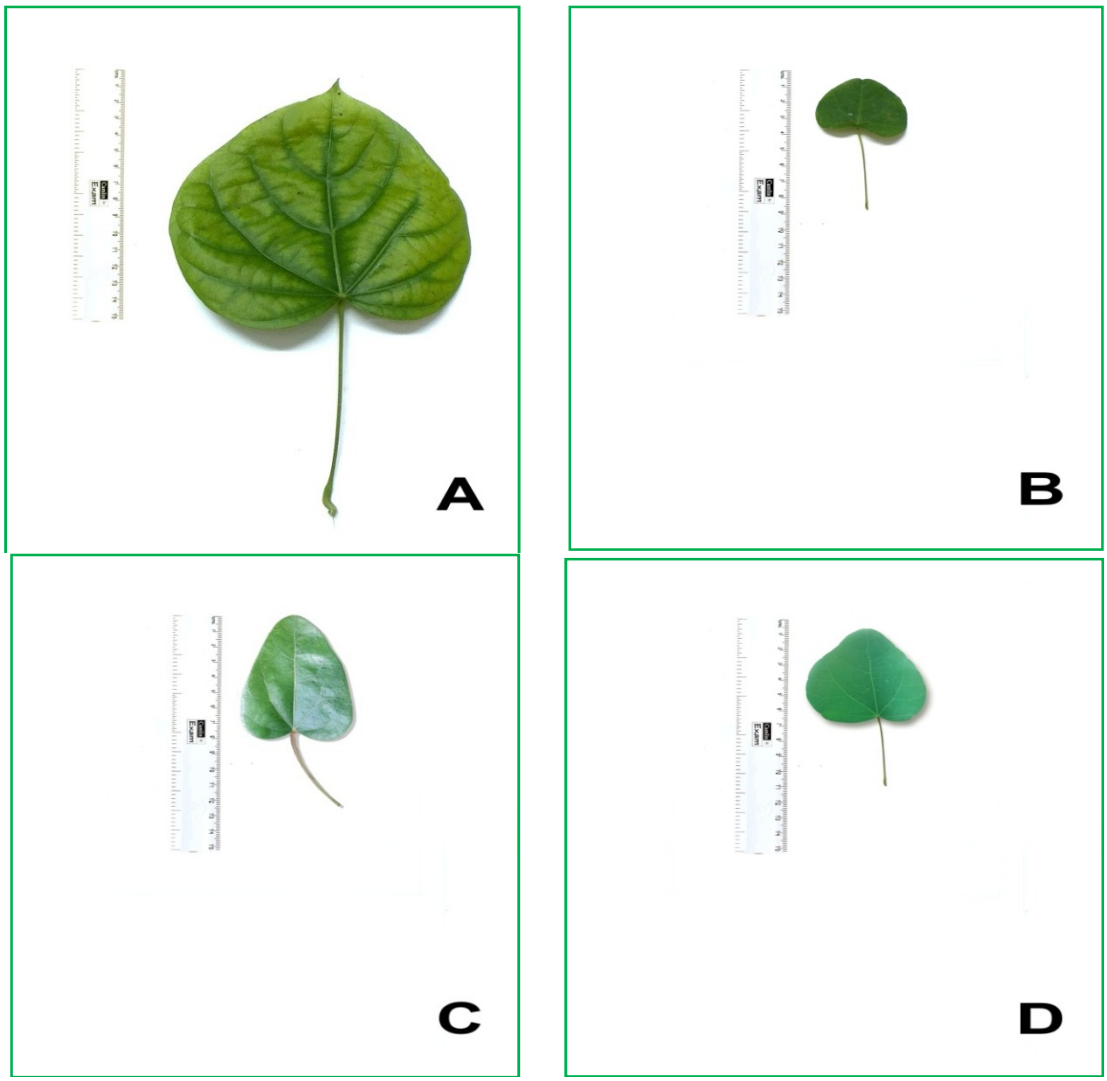

Figure 2: Leaf morphology A- ***Anamirta cocculus*****, B-** ***Cissampelos pareira*** **var** ***hirsuta*****, C-** ***Pachygone ovata*****, D-** ***Diploclisia glaucescens.***

Table 2: Comparative leaf morphological characters of selected plants

Sl No	Parameter	Observations			
		Anamirta cocculus	*Cissampelos pareira* var *hirsuta*	*Pachygone ovata*	*Diploclisia glaucescens*
1.	Leaf shape	Ovate	Peltate	Ovate -lanceolate	Round to kidney shaped
2.	Leaf apex	Acuminate	Rounded	Mucronate	Obtuse
3.	Leaf base	Cordate to truncate	Cordate, broader than longer	Obtuse rounded	Truncate
4.	Margin	Entire	Ciliate	Entire	Entire
5.	Venation	Palmate	Finely palmate	Cross venulate	Pinnate
6.	Leaf arrangement	Alternate spiral	Alternate spiral	Alternate distichous	Alternate spiral
7.	Hairs	Glabrous on both sides	Pubescent on both sides	Both upper and lower surface contain pale hairs	Pubescent on both sides
8.	Domatia	Domatia present as hairy patches in the axils of main and secondary vein	Domatia absent	Domatia absent	Domatia absent
9.	Petiole	Long glabrous petiole, swollen at both ends. Geniculate base	Long hairy petiole, inserted with in basal margin	Haired petiole Pulvinus present on petiole	Long and thin stalk, petiole usually longer than lamina
10.	Stipule	Absent	Absent	Absent	Absent

Table 3: Phytochemical screening of selected plants

S. No.	Name of the test	Chloroform				Methanol			
		A	B	C	D	A	B	C	D
1. a.	Carbohydrate test Fehlings test	+	+	+	+	-	-	-	-
b.	Molishs test	+	+	+	+	+	+	+	+
c.	Iodine test	+	+	+	-	-	-	-	-
2. a.	Protein test Biuret test	+	+	+	+	-	-	-	+
b.	Million test	+	+	+	+	+	+	+	+
3.	Aminoacid ninhydrin test	-	-	-	+	-	-	-	-
4.	Tannin and phenolic compound	-	+	+	+	+	-	+	+
5.	terpenoid	-	-	-	+	+	+	+	+
6. a.	Steroid Salkowski reaction	+	+	+	+	+	+	+	+
b.	Liebermanns reaction	+	+	+	+	+	+	+	+
7.	Glycosides keller-killani test	-	+	-	-	+	+	-	+
8.	Quinone	-	+	+	-	+	+	+	+
9.	Anthraquinone	-	-	-	-	+	+	-	+
10.	Saponin	+	-	-	-	-	-	-	+
11. a.	Alkaloid mayers test	+	+	+	+	+	-	-	+
b.	Hagers test	+	+	+	+	+	-	-	+
12.	Coumarin	+	+	+	+	+	+	+	+
13.	Flavanoid	+	+	+	+	+	-	-	-
14.	Resin	+	-	-	+	+	+	+	-

A- ***Anamirta cocculus*****, B-** ***Cissampelos pareira*** **var** ***hirsuta*****, C-** ***Pachygone ovata*****, D-** ***Diploclisia glaucescens***
+ presence, - absence

Table 4: Physico-chemical characterisation of selected plants

S. No	Name of the plant	Moisture content %	Total ash content %	Solvent used for extraction	Physical appearance of the extract	Consistency	Weight of the extract taken (g)	Yield%
1.	*Anamirta cocculus*	6.89	7.66	Chloroform	Reddish green	Waxy solid	0.543	3.623
				Methanol	Brown	Solid	0.866	7.217
2.	*Cissampelos pareira* var *hirsuta*	10.48	13.68	Chloroform	Brown	Solid	0.293	2.934
				Methanol	Deep Brown	Solid	0.279	3.487
3.	*Pachygone ovata*	8.23	6.17	Chloroform	Reddish Brown	Waxy solid	0.293	2.663
				Methanol	Reddish Brown	Semi solid	1.023	10.237
4.	*Diploclisia glaucescens*	9.78	8.63	Chloroform	Deep Brown green	Waxy solid	0.273	7.751
				Methanol	Reddish Brown	Semi solid	2.042	20.424

CONCLUSION

The study shows that Chloroform and Ethanol leaf extracts of selected plants contain medicinally improved bioactive compounds. This also justifies the use of these plants as traditional medicines. Studies on physicochemical and phytochemical screening can provide a

valuable source of information to determine the quality of the plant material in future investigations.

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