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Physicochemical and Phytochemical Screening of Hydroalcoholic Extract of Various Herbal Drugs with Anticancer Activity

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ABSTRACT

The aim of present study was to screen the physicochemical and phytochemicals of hydroalcoholic extract of various herbal drugs like Cajanas cajan, Curcuma longa, Piper nigrum, Glycyrrhiza glabra, Coriandrum sativum, Withania somnifera with anticancer activity. Considering the importance of the above mentioned plants an attempt is made to study the preliminary physicochemical and phytochemical screening. The study includes preparation of extract by cold maceration process for detailed analysis. Different physicochemical parameters like ash value (total ash and acid insoluble ash), loss on drying and phytochemical parameters such as extractive value (alcohol soluble and water soluble) are carried out as per recommended procedures. The phytochemical determinations revealed that all the selected plants contain alkaloids, flavanoids, tannins and glycosides.

Keywords: Phytochemical screening, Ash values, Extractive values

INTRODUCTION

Herb has various meanings, but in simple terms, it refers to "crude drugs of vegetable origin utilized for the treatment of diseases states, often of a chronic nature, or to attain or maintain a condition of improved health". Herbal preparations called, Phytopharmaceuticals, Phytomedicinal or Phytomedicine, are preparations made from different parts of herbs or plants [1]. They come into different formulations and dosage forms including tablets, capsules and elixir, powder, extract, tincture, cream and parental preparations. Natural products have played an eminent role in the discovery and development of new drugs [2].

The traditional medicine is largely gaining popularity over allopathic medicine because of the following reasons favourable to it, Rising cost of medicinal care, As these are from natural origin, so free from side effects, Goes to root cause and removes it, so that the disease does not occur again, Freedom from approaching various specialty, Easy availability of drugs from natural sources.

Due to the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities [2]. However, only approximately 5000 of the world's estimated 2,50,000-4,00,000 flowering plants have as yet been analyzed for their possible medicinal uses [3]. A recent estimate indicates that between 39-43% of endemic plant and vertebrate animal species in 25 ecological hotspots would become extinct by 2100 [4,5]. Hence, there is an urgent need to screen the medicinal plants for their biological properties and to isolate their active constituents. Therefore, in the present study, I am interested in screening the plants for their physicochemical and phytoconstituents.

MATERIALS AND METHODS

Plant material

The plant parts were collected from the local market of New Delhi. Plant parts were dried under shade for 15 days, separately powdered and stored in well closed airtight containers for further use. Powder prepared from plant parts purchased from local market of New Delhi was labeled as powder (1). Powder of the plants purchased from supplier was labeled as powder (2).

Extraction

The shade dried coarsely powdered plant parts were extracted with 80% aqueous methanol by maceration at room temperature for 72 h [6-10]. After completion of extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were then stored in desiccator. Hydroalcoholic extract prepared from plant parts was marked as extract (1) and purchased from supplier was marked as extract (2).

Physicochemical parameters

Ash value: For the determination of various ash values viz. total ash and acid insoluble ash all the powdered plant materials were passed through sieve no 40 and used.

Total ash: About 1 g of powdered drug was accurately weighed and taken in tarred silica crucible previously ignited and weighed, the powdered drug was scattered into a fine layer at the bottom of the crucible and incinerated gradually by increasing the heat not exceeding dull red heat, cooled and weighed. The charred mass was exhausted with hot water and the residue was collected on an ash less filter paper. The process was repeated until the successive weights differed not more than 0.5 mg (Ignition to constant weight). The percentage of ash with reference to air dried drug was calculated and tabulated in Table 1.

Table 1: Data showing total ash values for the powders and extracts

S. No.	Plant	Powder (% w/w)*		Extract (% w/w)*	
		1	2	1	2
1	*Cajanas cajan*	2.56	1.02	2.56	2.03
2	*Curcuma longa*	3.06	2.98	3.04	2.53
3	*Piper nigrum*	1.54	1.03	1.15	1.01
4	*Coriandrum sativum*	4.95	3.05	3.57	2.79
5	*Glycyrrhiza glabra*	4.91	3.41	4.08	3.76
6	*Withania somnifera*	3.44	2.92	3.43	3.12

Acid insoluble ash: The total ash was boiled for 5 min with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ash less filter paper wet with hot water ignited and weighed. The percentage of acid insoluble ash was calculated with reference to air dried drug and tabulated in Table 2.

Table 2: Data showing acid insoluble ash values for the powders and extracts

S. No.	Plant	Powder (% w/w)*		Extract (% w/w)*	
		1	2	1	2
1	*Cajanas cajan*	0.78	0.88	0.57	0.67
2	*Curcuma longa*	0.76	0.89	0.93	0.18
3	*Piper nigrum*	0.13	0.28	0.10	0.15
4	*Coriandrum sativum*	0.17	0.21	0.11	0.13
5	*Glycyrrhiza glabra*	0.86	0.12	0.28	0.32
6	*Withania somnifera*	0.45	0.58	0.77	0.82

Loss on drying: Glass stoppered shallow bottle was weighed that had been dried in the same conditions to be employed in the determination. About 1 g of the sample was transferred to the bottle, closed and accurately weighed. The samples were distributed as evenly as practicably gently side wise shaking to a depth not exceeding 10 mm. The loaded bottle was placed in a drying chamber (remove the stopper and left in the chamber). The sample was dried to a constant weight, the drying chamber was opened and bottle was closed and allowed to cool. The bottle and the contents were weighed. The process was repeated until the successive weights differed not more than 0.5 mg (Drying to constant weight). The percentage loss of weight was calculated and tabulated in Table 3.

Table 3: Data showing loss on drying values for the powders and extracts

S. No.	Plant	Powder (% w/w)*		Extract (% w/w)*	
		1	2	1	2
1	*Cajanas cajan*	3.71	5.43	3.67	5.25
2	*Curcuma longa*	4.29	5.64	3.05	5.13
3	*Piper nigrum*	3.81	4.29	6.18	5.06
4	*Coriandrum sativum*	4.19	5.71	4.32	5.76
5	*Glycyrrhiza glabra*	4.09	5.43	3.53	4.32
6	*Withania somnifera*	5.38	6.17	2.86	3.91

Phytochemical parameters [11-14]

Extractive value

Alcohol soluble extractive: About 5 g of dried coarse powder with 100 ml of 90% methanol was kept in a closed flask for 24 h, shakes

frequently during 6 h and allowed to stand for 18 h. Then it was filtered immediately to prevent loss of methanol. 25 ml of the filtrate was evaporated to dryness in a tarred shallow dish. The residue was dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug and tabulated in Table 4.

Table 4: Data showing alcohol soluble extractive values for the powders and extracts

S. No.	Plant	Powder (% w/w)*		Extract (% w/w)*	
		1	2	1	2
1	*Cajanas cajan*	13.96	11.82	22.17	19.29
2	*Curcuma longa*	18.48	16.02	25.82	21.76
3	*Piper nigrum*	13.36	10.75	19.97	17.61
4	*Coriandrum sativum*	11.15	9.66	16.56	14.18
5	*Glycyrrhiza glabra*	13.21	10.19	21.03	19.21
6	*Withania somnifera*	21.07	20.14	25.04	23.84

Water soluble extractive: 5 g of coarse powder of sample weighed and dissolved in 100 ml of water in a stoppered flask, heated at 80°C, shake well and allow to stand for 10 min, cool and add 2 g of kieselghur, filtered and transfer 5 ml of the filtrate to a tarred evaporating dish, evaporate the solvent on a water bath and the residue was weighed. The percentage of water soluble extractive was calculated with reference to air dried drug and tabulated in Table 5.

Table 5: Data showing water soluble extractive values for the powders and extracts

S. No.	Plant	Powder (% w/w)*		Extract (% w/w)*	
		1	2	1	2
1	*Cajanas cajan*	10.83	6.57	18.33	17.48
2	*Curcuma longa*	11.06	10.87	14.23	12.89
3	*Piper nigrum*	12.72	11.45	17.78	14.98
4	*Coriandrum sativum*	20.26	18.91	24.47	22.89
5	*Glycyrrhiza glabra*	26.31	21.06	27.06	25.41
6	*Withania somnifera*	10.67	6.81	12.76	11.9

Test for carbohydrates

Molisch's test: The extract was dissolved in 4 ml of distilled water and filtered. The filtrate was treated with 2-3 drops of 1% alcoholic α-naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Detection of fixed oils and fats

Saponification test**:** Few drops of 0.5 N alcoholic potassium hydroxide was added to small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 h. Formation of soap indicates the presence of fixed oils and fats.

Test for proteins and aminoacids

Ninhydrine test**:** The extract was treated with ninhydrine reagent. Purple colour produced shows the presence of proteins and free amino acids.

Test for phenolic compounds and tannins

Small quantity of the extract was taken in water and test for the presence of phenolic compounds and tannins was carried out with 5% Ferric chloride solution. Formation of violet colour shows the presence of tannins and phenolic compounds.

Test for alkaloids

Small quantity of extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filtrate was treated with Wagner's reagent. Formation of reddish brown precipitate shows the presence of alkaloids.

Test for flavanoids

Small quantity of extract was dissolved in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids. All the preliminary screening of phytoconstituents are evaluated and tabulated in Table 6.

Table 6: Data showing preliminary phytoconstituents screening for the extracts

S. No.	Plant	Alkaloids	Glycosides	Flavonoids	Tannins	Phenols	carbohydrates	Amino acids	Fixed oils and fats
1	*Cajanas cajan*	+	-	-	+	+	+	-	-
2	*Curcuma longa*	+	+	+	+	+	+	+	-
3	*Piper nigrum*	+	-	-	+	+	+	+	+
4	*Coriandrum sativum*	+	-	-	+	+	+	+	+
5	*Glycyrrhiza glabra*	-	-	-	-	-	-	-	-
6	*Withania somnifera*	+	-	-	-	-	+	+	+

RESULTS AND DISCUSSION

All the results obtained from the present study are represented in respective tables. The powdered plant parts and their extracts were subjected to preliminary physicochemical and phytochemical analysis which was found to be very promising. The percentage of total ash and acid insoluble ash were carried out and results are tabulated in Tables 1 and 2. The determination of ash value was carried out which gives an idea of the earthy material or inorganic composition and other impurities present along with the drug. The analytical results showed that total ash and acid insoluble ash values were found higher in all powders prepared in the laboratory.

Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies [13]. Moisture content values were found to be less in the samples prepared in laboratory. The powder and extracts prepared in the laboratory had shown higher extractive values than purchased from local supplier. The phytochemical determinations revealed that all the selected plants contain alkaloids, flavanoids, tannins and glycosides.

CONCLUSION

The present physicochemical and phytochemical screening of powdered plant material and hydroalcoholic extract of various herbal drugs like *C. cajan, C. longa, P. nigrum, G. glabra, C. sativum, W. somnifera* provide useful information regarding their identification. The phytochemical constituents which are screened could be used as diagnostic tool for the standardization of medicinal plants. There is an increasing awareness that many components of traditional medicine are beneficial and hence WHO encourages to identify and provide safe and effective remedies for use in public and private health services [12]. The study revealed that all the selected plants contain alkaloids, flavanoids, tannins and glycosides.

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