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Original Article

PHARMACOGNOSTICAL EVALUATION OF EMBELIA RIBES AND CAMELLIA SINENSIS: COMPARATIVE STUDIES OF THEIR ANTHELMINTIC ACTIVITY

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ABSTRACT

Objective: The present research work deals with the establishment of a standardization parameter for the Pharmacognositical evaluation of the plant Embelia Ribes and Camellia sinensis. Embelia Ribes is widely using in parasitic intestinal infection, however; Camellia sinensis is mainly used in the beverage preparation, for their Antioxidant activity but their anthelmintic effect is unknown. The present study aims to find out the effect of Camellia sinensis against pheritima Posthuma and compare their anthelmintic effect with Embelia Ribes.

Methods: The study has been performed under the different parameters 1). The physicochemical parameter includes total Ash value, acid insoluble ash value, water-soluble ash value, moisture content, and foreign organic matter 2). The phytochemical investigation includes the Extraction of Embelia Ribes and Camellia sinensis in different solvents in the increasing order of their polarity with Petroleum ether, chloroform, ethanol, distilled respectively and showed the presence of Alkaloids, carbohydrates, glycosides, tannins, flavonoids, phenolic compounds. 3) The pharmacological studies include the *In vitro* anthelmintic effect of Embellia Ribes and Camellia sinensis (ethanolic extract) against Pheritima Posthuma. The paralysis time and death time were studied.

Results: Shows the physic-chemical parameters such as total Ash value, acid insoluble ash value, water-soluble ash value, moisture content and foreign organic matter, which was determined to be not more than 8.98%, 1.5%, 7.8%, 3.2% and 0.25% in Embelia ribes as well as 7.78%, 1.6%, 8%, 2.2% and 0.21% in Camellia sinensis. Anthelemintic effect of Embellia ribes and Camellia sinensis (ethanolic extract) were investigated by measuring the paralysis time (75+3.76, 60+4.30, 48+4.40 and 73.32+3.80, 53.34+2.50, 38.55+3.45 respectively) and death time (140+2.82, 135+9.95, 97.55+4.25 and 124.85+5.30, 110.82+5.80, 80.30+2.80) at three different concentration 25 mg, 50 mg, 100 mg, against Pheritima posthuma and compares with the standard drug (Albendazole).

Conclusion: Camellia sinensis (ethanolic extract) extract and affords protection against helminthes (Pheritima Posthuma) and results show that Camellia sinensis is as effective as embellia ribes.

Keywords: Embelia ribes, Camellia sinensis, Antioxidant, Anthelmenitic, Pheritima posthuma

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INTRODUCTION

Embelia ribes Burm (family, Myrsinaceae), known commonly as vidanga [1] also known as Baibirang, is one of the important medicinal plants of India. Traditionally the seeds of Embelia ribes are employed as a remedy for a parasitic intestinal infection, toothache, headache, and snakebite. The seed is, round, red to black, resembles so much to pepper, and also known as false pepper [2]. The fruits are mainly used as astringent, carminative, stimulants, and to support the digestive function [2]. Camellia sinensis (family, Theaceae) is a tree that mainly grows in tropical and subtropical climates, whose leaves are the source of one of the most popular non-alcoholic beverages worldwide [3] it has been reported that Embelia ribs have the property to kill the helminthes, but the role of Camellia sinensis is unknown against helminthes Now these days the treatment for intestinal infection has been a challenge, which is currently being managed with drug combinations. Available therapies are too long and their sustained effect is negligible, further their withdrawal allows for the excystation of encysted parasites and relapse of the infection. However, they do not completely eradicate the parasite, either due to delayed treatment or resistance of the parasite to the treatment agent(s). The present study was designed to investigate the role of Camellia Sinensis against helminthes as it consumes and uses in non-alcoholic preparation more prominent.

MATERIALS AND METHODS

Drug and chemical

The entire chemicals were reagent grade and were purchased from CDH, New Delhi. Ethanol, Methanol, carbon tetrachloride, benzene, petroleum ether, Naphthyl ethylenediamine dihydrochloride, Sodium nitroprusside, Phosphate buffer saline, Glacial acetic acid, Ferric chloride, EDTA, Ascorbic acid, Vitamin E, Biochemical estimation kit, Carbon tetrachloride, Chloroform, Saturated solution of picric acid, Disodium hydrogen phosphate (NA2HPO4), Hydrogen peroxide (H2O2), Dihydrogen potassium phosphate anhydrous (KH2PO4).

Plant collection and authentication

Plant specimen Embelia Ribes seeds and Camellia Sinensis leaves were bought and further identified by Dr. Arnind Kumar, Associate Professor, Dept. of Pharmacy, SD College of Pharmacy and vocational studies, Muzaffarnagar, and deposited in the herbarium of Hemwati Nandan Bahuguna Garhwal university Uttarakhand with the herbarium Ref. no. BOT/EmRi/321 and BOT/CaSi/19 respectively

Extraction of *Embelia ribes* seeds

Soxhlet apparatus with n-hexane was used for the extraction coarsely powdered fruits of E. ribes. After 12 h of the solvent was evaporated using steam bath and petroleum ether was added to the residue and was filtered. After filtration, the remaining residue was mixed with cold petroleum ether in a separating funnel and it was again filtered. After that, filtrate residue was dried and crystallized with chloroform [5]

Extraction of Camellia Sinensis leaves

The fresh plants were brought from Attar market Muzaffarnagar District, Uttar Pradesh India. The plant was recognized as Camellia sinensis in the Dept. of Pharmacy, SD College of pharmacy and vocational studies,

Muzaffarnagar, and the herbarium was registered as BOT/CaSi/19. The fresh young leaves were dried in shades at room temperature for 10 d and powdered to obtain 2-3 mm particle sizes. Fifty grams of powdered plants were macerated in 1,500 ml hydro-alcoholic solution (50% water+50% absolute ethanol) for 72 h. The extracts were filtered and concentrated in a rotary evaporator to obtain solid extracts and then freeze-dried to remove the solvent completely [6].

Physico-chemical analysis: The coarse powder of Embelia Ribes seeds and Camellia sinensis leaves were subjected to various Physico-chemical studies for determination of ash value (total ash, acid insoluble, and water-soluble ash) extractive values (water-soluble, alcohol soluble and petroleum ether soluble, and chloroform-soluble) respectively.

Preliminary Phytochemical screening

Qualitative estimation of hydroalcoholic extract of Embelia Ribes and Camellia sinensis was performed for the identification of various chemical constituents like alkaloid, carbohydrate, flavonoids, proteins, amino acids, phenols, tannins, glycosides and steroids [12].

Pharmacognostic Investigation

Preliminary Pharmacognositical study on the leaves of Camellia sinensis and seeds of Embelia Ribes studied to determine various parameters of Pharmacognositical standards such as ash values, extractive values, phytochemical tests, and microscopically characters of leaf powder. The shade-dried powder and various solvent extracts (viz., 70% ethanol, chloroform, and petroleum ether) have been analyzed for their phytoconstituents. The petroleum ether and chloroform extract were found to contain the presence of terpenoids and steroids. The data generated for the evaluation on Camellia sinensis leaves may be useful for establishing the standardization protocols [7].

Pharmacognostic and physic-chemical standardization of ethno pharmacologically important seeds of Embelia Ribes and leaves of Camellia sinensis were performed. The physicochemical standards developed in this study will provide referential information for the identification of these crude drugs and standardization. Quality control standardizations of the various medicinal plants used in traditional medicine are becoming more important today given the commercialization of formulations based on these plants. E. ribes and Camellia sinensis seeds and leaves evaluated as per WHO recommendation respectively, various physicochemical and phytochemical evaluation parameters for quality control of medicinal plants are performed. Because of their medicinal importance and taxonomic confusion, Physico-chemical parameters, preliminary phytochemical screening, and quantitative estimation were performed to establish the salient diagnostic characters [8].

Pharmacological evaluation

Procedure

The anthelmintic activity was performed according to the method of Gosh *et al.* (2005) on the adult Indian earthworm Pheritima Posthuma. Albendazole, the standard drug, was diluted with normal saline to obtain 25, 50, and 100 mg/ml concentrations and was poured into Petri dishes. Ethanolic extracts of both plants were diluted with normal saline to obtain 25, 50, and 100 mg/ml concentrations respectively. Normal saline (0.9% NaCl) alone served as the negative control.

All these dilutions were poured into the Petri dishes accordingly. Six groups of earthworms (n = 6) were taken for the study. Earthworms, nearly equal sizes (about 8 cm), were placed in each Petri dish at room temperature. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. The time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). The paralysis time and lethal time were recorded in terms of minutes [10]

RESULTS

Physicochemical studies

Embelia ribes seeds

The physicochemical parameters total Ash value, acid insoluble ash value, water-soluble ash value, moisture content, and foreign organic matter which was determined to be not more than 8.98%, 1.5%, 7.8%, 3.2%, and 0.25% respectively shows in table 1. The extractive values (petroleum ether, chloroform, ethanol, Alcohol, Distilled water) were determined to be not more than 2.4%, 2.8%, 3.2%, 11.62%, and 7.5% respectively shows in table 2)

Camellia sinensis leaves powder

The physicochemical parameters total Ash value, acid insoluble ash value, water-soluble ash value, moisture content, and foreign organic matter which was determined to be not more than 7.78%, 1.6%, 8%, 2.2%, and 0.21% respectively (table 1). The extractive values (petroleum ether, chloroform, ethanol, Alcohol, Distilled water) which were determined to be not more than 2.5%, 2.7%, 3.5%, 11.98% and 7.9% respectively (table 2)

Preliminary phytochemical investigation

Extractive values using successive extraction successive extraction was performed by Soxhlet apparatus with different solvents in the increasing order of their polarity e. g. Petroleum ether, chloroform, ethanol, distilled water and percentage yield is calculated (table 3). The foaming index was less than 100; although the Swelling Index of Embelia ribes and Camellia sinensis was found to be 1.44 and 1.10 respectively.

DISCUSSION

In the present study, varied Pharmacognositical and chemical science parameters like ash values, extractive values, and wetness content of Embelia ribes seeds and Camellia sinensis were established. These parameters play a very important role within the determination of the identity, quality, and purity of the drug. Wetness in conjunction with an acceptable temperature result in the activation of enzymes and enhances for the expansion of micro-organisms that could be a major issue liable for the deterioration of the medicine and formulations. Swelling index is that the property of plant containing gums, mucilage, or hemicelluloses, which can be liable for specific therapeutic utility whereas foaming index determines the glucosidal content of the drug [14]. Total ash indicates the presence of inorganic salts like phosphates, carbonates, and silicates of metallic element, potassium, magnesium, calcium, etc. Acid insoluble ash obtained when boiling the ash with robust acid-like acid and igniting the insoluble portion. It offers a live of sand and alternative oxide matter. Water-soluble extractive worth shows the presence of water-soluble inorganic salts. Phytochemical standardization encompasses all potential data concerning the chemical constituent's gift in associate flavorer drug. This study discovered the presence of vital phytochemical contents such Alkaloids, carbohydrates, glycosides, tannins, Flavonoids, phenolic resin from the tested plants and compare their anthelmentic activity. Tannins and Phenolics are familiar to interfere with the energy generation in parasitic worm parasites by uncoupling biological process [13]. The Petroleum ether and chloroform extract were found to contain the presence of terpenoids and steroids. The info generated for the analysis on shrub leaves is also helpful for establishing the standardization protocols. Shrub is wide used, for their antioxidant activity. Antioxidant molecules have the capability to manage or neutralize the free radicals [12]. The leaf extract of shrub has important inhibitor and anthelmintic activity. However any investigations on the isolation of active compounds gift within the extracts and *in vivo* studies are necessary to spot a possible chemical entity for clinical use.

CONCLUSION

The pharmaognositcal characterstics were evaluated successfully by using the different solvent extract of E. ribes and camellia sinensis. the method of evaluation was simple and inexpensive to obtain the optimized form. Fig. 1 shows the camparison of E. Ribes and C. sinensis with the standard drug (Albendazole). It indicates the significant change in the death time of pheritima posthuma as we increase the concentration of both tested drugs. It also provides evidence, that C. sinensis as effective as Albendazole as and more effective than E. Ribes. Fig. 2 shows the plot between paralysis time and different concentrations of ABZ, E. Ribes and C. sinensis respectively. it shows that there is no significant change at low concentration (25 mg/dl) in

paralysis time but it has been found as we increase the concentration of tested drug it shows the significant change and in paralysis time. Camellia sinensis (ethanolic extract) extract affords maximum protection against helminthes (Pheritima Posthuma) and results show that Camellia sinensis is more effective than Embellia ribes.

Table 1: Ash valve of Embelia ribes seeds and camellia sinensis leaves

Solvents	%value (w/w) Embelia ribes	%value (w/w) camellia sinensis
Petroleum ether	2.4	2.5
Chloroform	2.8	2.7
Ethanol	3.2	3.5
Distilled water	7.5	7.9

Table 2: Extractive values of embelia ribes and camellia sinensis

Parameter	%Value(w/w) Embelia ribes	%Value(w/w) camellia sinensis
Total ash	8.98	7.78
Acid insoluble ash	1.5	1.6
Water-soluble ash	7.8	8
Moisture content	3.2	2.2
Foreign organic matter	0.25	0.21

Table 3: Swelling index and foaming index of Embelia ribes and Camellia sinensis

Parameter	Value (Embelia ribes)	Value (Camellia sinensis)
Swelling index	1.44	1.10
Foaming index	Less than 100	Less than 100

Table 4: Phytochemical analysis showed the presence of Alkaloid, carbohydrate, glycosides tannins, flavanoids, and steroids in the extract

Phyto-chemical	PEL.	CEL	ETH
Alkaloids-	–	–	+
Carbohydrates-	–	–	+
Glycosides-	–	–	+
Protein and amino acids-	–	–	–
Tannin-	–	–	+
Terpenoids	+	+	–
Saponin	–	–	–
Flavonoids	–	–	+
Steroids-	–	–	+

PEL: pet ether extract of *Embelia ribes Seeds*; CEL: chloroform extract Embelia ribes *Seeds*; ETH: Ethanol extract of Embelia ribes *Seeds*;

Table 5: Phytochemical analysis showed the presence of Alkaloid, carbohydrate, glycosides tannins, flavonoids, and steroids in the extract

Phyto-chemical	PEL.	CEL	ETH
Alkaloids-	-	-	-
Carbohydrates-	–	–	+
Glycosides-	–	–	–
Protein and amino acids-	–	–	–
Tannin-	–	–	+
Terpenoids	+	+	–
Saponin	–	–	–
Flavonoids	–	–	+
Steroids-	–	+	+

PEL: pet ether extract of Camelia sinensis leaves; CEL: chloroform extract Camelia sinensis leaves; ETH: Ethanol extract of Camelia sinensis leaves

Anthelmintic activity

Table 6: *In vitro* anthelmintic effect of Embellia ribes and Camellia sinensis (ethanolic extract) against pheritima posthuma

Drugs (treatment)	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Albendazole(standard)	25	55.44+3.40	120.82+7.0
	50	40.66+4.50	98.6+4.89
	100	36.55+3.58	75.85+4.28
Embelia ribes	25	75+3.76	140+2.82
(ethanolic extract)	50	60+4.30	135+9.95
	100	48+4.40	97.55+4.25
Cambellia sinensis	25	73.32+3.80	124.85+5.30
(ethanolic extract)	50	53.34+2.50	110.82+5.80
	100	38.55+3.45	80.30+2.80

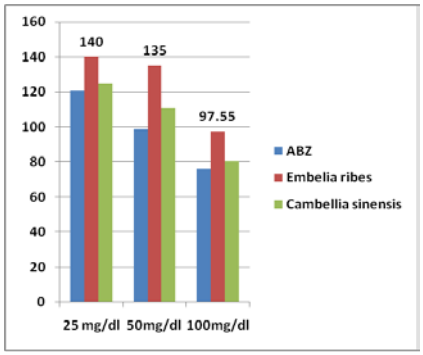

Fig. 1: Death time (minute) verses concentration (25, 50, 100 mg/dl) of Ablbendazole (ABZ), Embelia ribes, Camellia sinensis

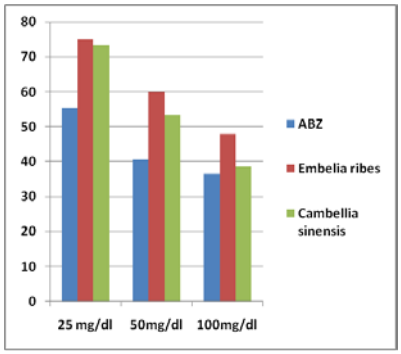

Fig. 2: Paralysis time (min) verses concentration (25, 50, 100 mg/dl) of Ablbendazole (ABZ), embelia ribes, camellia sinensis

ABBREVIATION

ABZ-Ablbendazole

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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