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PRELIMINARY PHYTOCHEMICAL SCREENING OF *CAMELLIA SINENSIS* & *TINOSPORA CORDIFOLIA* USED IN TRADITIONAL MEDICINE

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ABSTRACT

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. In the present study, chief phytoconstituents of the two selected medicinal were identified in order to relate their presence with bioactivities of the plants. Screening of two selected medicinal plants (*Camellia sinensis* & *Tinospora cordifolia*) was performed for the presence of tannins, flavonoids, terpenoids, steroids, glycosides, alkaloids and phenolic compounds using standard methods. The selected medicinal plants were found to contain tannins, flavonoids, phenolic compounds, terpenoids, glycosides, alkaloids in different extracts of methanol, diethyl ether, isoamyl alcohol, water, butan1-ol, ethyl acetate in *Camellia sinensis* and *Tinospora cordifolia*. Moreover, flavonoids were also present in water and methanol extracts of *Tinospora cordifolia* and *Camellia sinensis*. On the other hand, alkaloids and steroids were present in the water, isoamyl alcohol, methanol extracts of *Camellia sinensis* and *Tinospora cordifolia*. In addition, tannins, glycosides and phenols were present in diethyl ether, butan1-ol, water extracts of *Camellia sinensis* and *Tinospora cordifolia*. It is evident from the study that *Camellia sinensis* & *Tinospora cordifolia* have the highest therapeutic efficacy possessing majority of phytochemical classes of compounds.

KEYWORDS: ***Camellia sinensis*, *Tinospora cordifolia*, Screening, Phytochemicals.**

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INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as antiviral drugs [10] antimicrobial drugs [33] and antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [5]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, glycosides, phenolic, steroids and flavonoids [12].These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites were chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [48].A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [8]. Plant products have been part of phytomedicine since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [9]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances. ***Camellia sinensis*** is consumed worldwide and is known as Green tea as a beverage and has described many health benefits such as reduction in cholesterol and protection against cardiovascular disease [37]. Green tea is generally safe, nontoxic and has no side effects after consumption [13]. The most important bioactive compounds present in *Camellia sinensis* leaves are alkaloid, flavonoids, steroids and terpenoids which serve as valuable starting material for the medicine development [22]. *Camellia sinensis* possess an antimicrobial activity because of the bioactive compounds [31]. The plant materials have shown the antimicrobial activities against various pathogenic micro-organisms therefore consumption of tea has been associated with reduced risk of major diseases [40]. The beneficial effects of the tea have been attributed to the strong antioxidant activity due to the phenolic compounds [17]. The carotenoids, flavonoids, benzonic acid, ascorbic acid, tocotrienols, cinnamic acid, folic acid, tocopherols are some antioxidents produced by the plants for their substance [6]. *Tinospora cordifolia* which is also known as Giloy belongs to the family Menispermaceae. Piles problem can be controlled by eating this plant mixed with milk or water and thus, preventing the bleeding and constipation [20]. The stem is bitter stomachic, diuretic [28] stimulates bile secretion, causes constipation, allays (satisfies) thirst, burning sensation, vomiting, enriches the blood and cures jaundice. The extract of stem is useful in skin diseases [4,36]. Its root and stem are prescribed in combination with other drugs as an antidote in snake bite and scorpion sting [27,50]. Oral administration of an *aqueous T. cordifolia* root extract to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids [11]. It is reported to benefit the immune system in a variety of ways [19]. Its hepatoprotective action was reported in one of the experiment in which goats treated with *T. cordifolia* have shown significant clinical and hematobiochemical improvement in CCl_4 induced hepatopathy. Extract of *T. cordifolia* has also exhibited in vitro inactivating property against Hepatitis B and E surface antigen [26]. Its aqueous extract exerted a significant anti-inflammatory effect on cotton pellet granuloma and formalin induced arthritis models [16]. In a clinical evaluation, a compound preparation Rumalaya containing *T. cordifolia* was reported to significantly reduce the pain in patients suffering from Rheumatoid Arthritis. In the present work, phytochemical analysis were carried in two medicinal plants -*Tinospora cordifolia* (Stem) were collected from Sodpur region of West Bengal and *Camellia sinensis* (Leaves) were collected from Palampur region of Himachal Pradesh.

MATERIALS AND METHODS

Collection of plant materials

The medicinal plants- *Tinospora cordifolia* (Stem) (Authentication No. GEC/357/2015) were collected from Sodpur region of West Bengal (Fig 1) and *Camellia sinensis* (Leaves) (Authentication No. GEC/357/2015) were collected from Palampur region of Himachal Pradesh (Fig 2). The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using a mechanical blender into fine powder and transferred into airtight containers with proper labelling for future use.

Figure 1
Powder of Tinospora cordifolia

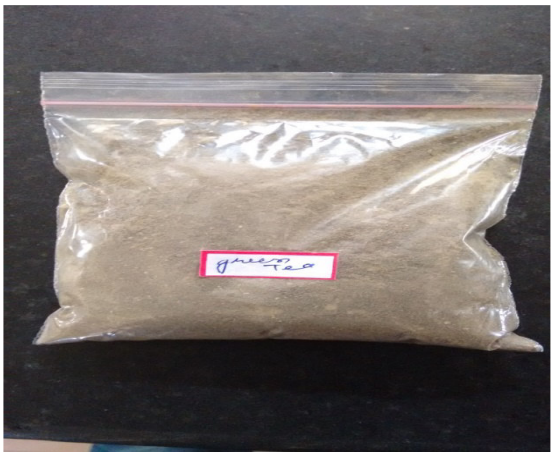

Figure 2
Powder of Camellia sinensis

Preparation of plant extracts
Hot water extraction

5gm of dried finely powdered plant material was taken in a beaker and 200ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 30^{o} -40^{o}C for 20 minutes (Fig 3). Then the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis. The water extract was kept in refrigerator when not in use.

Figure 3
Water bath Instrument

Solvent extraction

Crude plant extract was prepared by Soxhlet extraction method (Fig 4). About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents were used methanol, ethyl acetate, isoamyl alcohol, diethyl ether, butan1-ol and water. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on a hot plate and heated at 30-40^{0}C till all the solvents got evaporated. Dried extract was kept in refrigerator at 4^{0}C for their future use in phytochemical analysis.

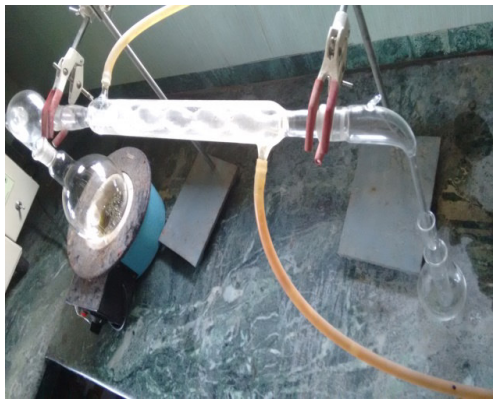

Figure 4
Soxhlet extraction apparatus

500 gm of collected leaves of *Camellia sinensis* and *Tinospora cordifolia* were air dried under a shade for two weeks and then crushed to coarse powder. The powdered leaves were extracted with solvents of different polarities. Methanol, ethyl acetate, isoamyl alcohol, diethyl ether, butan1-ol and water were used by cold maceration method [14]. The powdered dry leaves of the two plants were separately soaked in the above solvents at room temperatures for 1 hour in rotary shaker. After the completion of extraction, the supernatants were filtered through Whatman No.1 filter paper (Fig 5). The filtrate obtained was kept in serological water bath at 40°C using Multispan (Ucon, Kolkata). The obtained semi solid extract was stored at 4°C in the refrigerator for qualitative phytochemical assays [21]. The extraction and phytochemical screening were done in duplicates.

Figure 5
Filtration after methanol solvent mixing

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods [43,47,15].

Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids [41].

Test for tannins

Crude extract was mixed with 2ml of 0.1% solution of $FeCl_3$. A brownish green or blue- black coloration indicated the presence of tannins [34].

Test for flavonoids Shinoda test
Crude extract was mixed with a few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids [7].

Test for glycosides Keller-Kilani test
Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides [32].

Test for phenols
Crude extract was mixed with 2ml of 5% solution of $FeCl_3$. A blue-green coloration indicated the presence of phenols [34].

Test for steroids
Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids [39].

Test for terpenoids
Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids [39].

RESULTS & DISCUSSION

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, antiinflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc [30]. From the above results, it can be noted that successful extraction of biologically active compounds from plants like *Camellia sinensis* and *Tinospora cordifolia* are largely dependent on the type of solvent used during extraction. Different solvents with differing polarities extract specific phytochemicals in plants [46]. In this study, polar solvents like water, methanol, ethyl acetate, isoamyl alcohol, diethyl ether, butan1-ol yielded the highest amount of crude extracts and also had the highest presence of phytochemicals. This study therefore validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract [29]. It also implies that the choice of a solvent is affected by different factors like class of phytochemicals, diversity and polarity of the compounds to be extracted [18].

Table 1
Phytochemical constituents of Tinospora cordifolia in water and different solvents extraction

Phytochemical test	Water	Isoamyl alcohol	Methanol	Ethyl acetate	Diethyl ether	Butan1-ol
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	-	+	-	-	+
Glycosides	+	+	-	+	+	+
Phenolic	+	+	+	+	-	+
Steroids	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	+

From these results, extracts of methanol, ethyl acetate, isoamyl alcohol, diethyl ether, butan1-ol, water indicated the presence of steroids in giloy and extracts of methanol, diethyl ether indicated the absent of steroid in green tea . Terpenoids have been obtained by successive extraction of dried barks with ethyl acetate, isoamyl alcohol, diethyl ether, butan1-ol, water in *Tinospora cordifolia* (Table 1) but extract of diethyl ether indicate absent of terpenoid in *Camellia sinensis* (Table 2). Occasionally tannins and terpenoids will be found in aqueous phase, but they are more often obtained by treatment with less polar solvents [46]. Steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune response [42].

Table 2
Phytochemical constituents of Camellia sinensis in water and different solvents extraction

Phytochemical test	Water	Isoamyl alcohol	Methanol	Ethyl acetate	Diethyl ether	Butan1-ol
Alkaloids	+	+	+	-	+	+
Tannins	+	+	+	-	+	+
Flavonoids	+	+	+	-	+	+
Glycosides	-	+	-	-	+	+
Phenolic	+	+	+	-	+	+
Steroids	-	+	-	+	-	+
Terpenoids	+	+	+	+	-	+

Qualitative screening of *Camellia sinensis and Tinospora cordifolia* showed that the leave extracts of the two medicinal plants have alkaloids,flavonoids, phenols, tannins, steroids and terpenoids. This variation in phytochemicals makes plants potential medicinal plants [25]. Investigation of alkaloids have revealed many pharmacological properties including antidiabetic, antiprotozoal and cytotoxic [1] and anti-inflammatory properties [23]. Flavonoids and phenolic compounds in various plants have been reported to have multiple

biological effects like antioxidant, free radical scavenging abilities, anti-inflammatory and anticarcinogenic properties [45]. The extracts of methanol, butan1-ol, water indicated the presence of flavonoids in giloy, but absent in ethyl acetate, isoamyl alcohol, diehyl ether and extracts of ethyl acetate indicated the absent of flavonoids in green tea. Steroids in plants are known for their insecticidal, analgesic properties, cardiotonic and central nervous system activities, immunomodulatory properties [49,35], antimicrobial and antiinflammatory properties [45]. Tannins isolated from medicinal plants have also been reported to exhibit remarkable toxicity against bacteria and fungi [2]. Terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well [44].

CONCLUSION

Results showed that methanol, ethyl acetate, isoamyl alcohol, diehyl ether, butan1-ol and water solvents are important in the extraction of polar phytochemicals and thus crucial in isolation of alkaloids, flavonoids, phenols and tannins, glycosides, terpenoids, steroids. Therefore, extracts from these plants in different solvents could be seen as a good source of bioactive compounds using extraction techniques.

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