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Reena Sinku
P.G. Department of Botany, Kolhan University, Chaibasa, Jharkhand, India

Manoj Ranjan Sinha
Department of Botany, Jamshedpur Co-operative College, Jamshedpur, Jharkhand, India

Correspondence
Reena Sinku
P.G. Department of Botany, Kolhan University, Chaibasa, Jharkhand, India

Preliminary phytochemical screening and physiochemical analysis of *Tinospora cordifolia* Miers

Reena Sinku and Manoj Ranjan Sinha

Abstract
The aim of this study was to attempt preliminary phytochemical screening and evaluate the therapeutic activity of extract of *Tinospora cordifolia* Meirs. Four different solvents viz; water, ethanol, methanol and acetone were used to obtained extracts of leaves and stems. These extracts were subjected for qualitative phytochemical analysis by using standard methods. The solubility percentages of alcohol is higher than water. Data indicate the presence of carbohydrates, proteins, alkaloids, flavanoids, saponins, glycosides, steroids, terpenoids, phenolics and tannins.Potential therapeutic properties of the plant reported by modern scientific research include antioxidant, anti-stress, anti-inflammatory, anti-allergic, anti-rheumatic, adaptogenic, antipyretic, antimalarial, anti-leprotic, hypoglycemic, hepatoprotective, immunomodulatory and anticancerous activities. The overall investigational results suggests that the bioactive compounds obtained from phytochemical screening may be responsible for the significant therapeutic activity.

Keywords: Antipyretic, antioxidants, adaptogenic, immunomodulatory

Introduction
In recent times, focus on plant research has increased all over the world and large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [1]. Tinospora cordifolia is one such plant which is widely used in indigenous system of medicine [2]. It belongs to the family Menispermaceae. It is a glabrous, succulent, climbing, shrub, often reaching a great height and sending down long thread like aerial roots [3]. Glabrous, cordate leaves, 2-4" or rarely 5.5" by 4.75", and rather lax racemes, 2", elongating and ultimately often longer than the leaves, racemes of green and scarlet drupels in fruit (4 Haines).

The plant has been used in traditional medicine since ancient times for its ability to impart youthfulness, vitality and longevity. For these qualities these are referd to as Amrita [5]. It has been widely used in the traditional system of medicine to cure diabetes, jaundice, rheumatism, bone fracture, fever, leprosy, asthama, anorexia, pyreia, gout and diarrhoea. The plant is categorized as a "Rasayana" in Ayurveda, it is used for its general adaptogenic and prohost immunomodulatory activity in fighting infections [6]. It possess anti- androgenic activity [7]. It has reported non- toxic in an acute toxicity [8]. It improves cognition, learning and memory in memory deficient rats [8]. Recently it has been extensively studied and reported to have potent antioxidant activity [9]. Antioxidants are known to prevent neurodegeneration [10]. Antioxidants are reported to regulate oxidative damage and other metabolic processe [11]. ECD (Epoxy Clerodane diterpene) exhibits preventive effect against chemically induced hepatocellular carcinoma [12]. When administered during the pre-operative period to patients with obstructive jaundice, it was found to decrease the post operative morbidity and mortality (due to sepsis and liver cell failure). It protected against ethanol (40%) induced toxicity in isolated rat hepatocytes. The plant also shows immunomodulatory and adaptogenic activity against several diseases [1]. Anti- pakinsonic activity was reported in ethanolic extract of the plant (TCEE). Parkinsonism was induced by stereotactic injection of 6- hydroxyl dopamine (6- OHDA) into the striatum of the brain, 6- OHDA is one of the most widely used model of PD in rats, it produces neuronal degeneration and enhances oxidative stress due to dopaminergic toxicity [13]. Reports shows that it induces apoptosis in malignant cells but protected the normal bone marrow from apoptosis induced by cyclophosphamide [14].

Plant Materials

The leaves, stems and tubers were collected from west singhbhum and surrounding areas from July to December 2016. Samples were authenticated by Dr. Kiran Shukla (H.O.D. University Department of Botany, Kolhan University, Chaibasa, Jharkhand). The voucher specimens were deposited in the University Department of Botany, Kolhan University, Chaibasa, Jharkhand. The samples were washed with tap water to remove dust and contaminant. The plant samples were shade dried until all the moisture evaporated and pulverized by using mechanical grinder and stored in air tight jar for further use.

Extraction of plant material

The plant materials were extracted with ethanol using sohxlet apparatus continuously for 6 to 8 hours. 50 gm of dried plant material was packed in filter paper and loaded into the thimble of sohxlet apparatus. 250 ml of different extract viz; aueous, ethanol,methanol and acetone was poured into the flask and the all apparatus was set. The extraction was performed for 6-8 hours. Later the extracted solvent was evaporated under reduced pressure. Then the extract was kept in refrigerator for further use.

Phytochemical Screening

Chemical test were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harbone

Extractive Value

About 5gm of air dried coarsely powdered sample of plants were accurately weighed and macerated for 24 hours with 100 ml of solvent (Ethanol) in a separate conical flasks with stopper. The flasks were then shaked in Magnetic stirrer for 40 minutes and then allowed to stand for 24 hours. Extract were filtered rapidly, 25 ml of extract was transferred in China dish and evaporated to dryness on water bath. The dried extract was further dried in hot air oven to constant weight at 105^0C, cooled in desiccators and weighed. The percentage of extractive values of different solvents was calculated using formula given below :

Extractive Value (% w/w) = [(Weight of residue x 100) / (25 x Weight of sample)] x 100

Moisture Content

5gm air dried coarse powder of sample were accurately weighed in previously tarred crucible and dried at 105^0 C in hot air oven to constant weight and cooled in desiccators. Percentage of moisture content was calculated using the expression given below :

$$\text{Moisture content (\% w /w)} = \frac{\text{Difference in weight before and after drying}}{\text{Weight of the sample before drying}} \text{ X } 100$$

Qualitative phytochemical screening

Test for proteins

Millon's Test

Crude extract when mixed with2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin Test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates

Fehling's Test

Equal volume of Fehling A and Fehling B reagent were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's Test

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrate.

Iodine Test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple colouration indicated the presence of the carbohydrate.

Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$.A blue- green or black colouration indicated the presence of phenols and tannins.

Test for flavonoids

Shinoda Test

Crude extract was mixed with few fragments of magnesium ribbon and conc. HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Alkaline reagent Test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Liebermann's Test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully conc. H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Sakowaski's Test

Crude extract was mixed with 2ml of chloroform. Then 2ml of conc. H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e.,glycone portion of glycoside.

Keller-Kilani Test

Crude extract was mixed with the 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 conc. H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for steroids

Liebermannan's Test

2ml extract was mixed with 2ml of glacial acetic acid. Solution was cooled followed by the addition of few drops of

conc. H_2SO_4. Colour development from violet to blue or bluish green was taken as positive test of steroidal ring.
Crude extract was mixed with 2ml of chloroform and conc. H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroid.
2ml crude extract was mixed with 2ml of chloroform. Then 2ml of each of conc. H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish colouration indicated the presence of steroids.

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

Test for phlobatanine
Crude extract was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatanine.

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

Result and Discussion

Table 1: Qualitative analysis of *Tinospora cordifolia* plant extracts

Name of compounds	Name of test	Leaf				Stem			
		Aqueous ext.	Ethanol ext.	Methanol ext.	Acetone ext.	Aqueous ext	Ethanol ext.	Methanol ext.	Acetone ext.
Proteins	Millon's test	+	-	+	+	+	+	+	+
	Ninhydrin test	+	+	+	+	+	+	+	-
Carbohydrates	Fehling's test	+	+	-	-	-	+	+	+
	Benedict's test	-	-	-	-	-	-	+	-
	Iodine test	-	-	-	-	-	-	-	-
Phenols and tannins	2ml ext.+ 2% $FeCl_3$	-	-	+	-	-	+	+	-
Test for flavonoids	Shinoda test	-	-	-	-	-	+	+	-
	Alkaline reagent test	+	-	-	+	-	+	+	+
Test for saponins	Foam test	+	+	-	-	+	-	-	-
Test for glycosides	Liebermann's test	+	+	+	-	+	+	+	-
	Salkowski's test	+	+	+	-	+	+	+	-
	Keller- Kilani test	+	+	+	-	+	+	+	-
Test for steroids	Liebermann's test	-	+	+	-	-	-	+	-
	2ml ext.+ 2ml chlo + conc. H_2SO_4	+	+	+	+	-	-	-	-
	2ml ext.+ 2ml chlo + conc. H_2SO_4 + acetic acid	+	+	+	-	+	+	+	-
Test for alkaloids	2ml ext. + 2ml 1%HCl + Mayer's + Wagner's reagents	+	+	+	+	-	+	+	+
Test for phlobatanine	2ml ext.+1ml 1% HCl	-	-	-	-	-	-	-	-
Test for terpenoids	2ml ext. + 2ml chlo + conc. H_2SO_4	+	+	+	+	+	+	+	-
Abbreviation chlo: chloroform, ext.: extract, + : present, - : absent									

Extractive Value

SL.	Stem	Extractive Value % WW
	Extract	
1	Aqueous	21.53%
2	Ethanol	20.03%
3	Petrolium ether	18.99%
	Leaf	
	Extract	
1	Aqueous	20.98%
2	Ethanol	21.25%
3	Petrolium ether	19.00%

Moisture content in % WW
STEM: 0.98%
LEAF: 0.85%
The preliminary phytochemical investigation of stem and leaves of *Tinospora cordifolia* revealed that the presence of above bioactive compounds (in table 1) exhibit great deal of medicinal importance like saponins act as anti- bacterial and antineoplastics [24]. Saponins has been also shown to exhibit apoptotic effect on cacer cells [14]. Flavonoids shows anti-inflammatory and antioxidant properties [14]. Glycosides and terpenoids is used in Alzeimer's disease [14]. Cytotoxic proteins and polypeptides that have been shown to induce tumour necrosis, increase natural killer cell activity, increase the production of interleukins 1 and 6, activate macrophages, induced programmed cell death and protect DNA in normal cells during chemotherapy [14]. Terpenoids used in hepatocacer therapy [12]. Amino acids and proteins indirectly influence antioxidant defense and oxidative damages [11]. Tannins and phenolics showed effective free radical scavenging activity [21]. Photoberberine and aporphine alkaloids have been reported from plant [19]. Medicinal value of the plant is due tosmall quantity of berberine [3]. Flavonoids show anti- allergic, anti-inflammatory and anti- cacer activity and alkaloids possess a good analgesic, anti- inflammatory

and anti- oxidant activity [24]. Several studies confirmed that these phytochemicals contribute in the treatment of different ailments [24].

Conclusion

The study on *Tinospora cordifolia* suggest a huge biological potential of this plant on the basis of that phytoconstituents which may supply novel medicines.The phytochemical variations and efficacy of the medicinal value of *Tinospora cordifolia* is depedent on geographical locations and seasons. Further exploitation of various extracts of *Tinospora cordifolia* could be in the future may develop new specialized drugs with more efficacy.

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