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Research Article

COMPARATIVE QUALITATIVE PHYTOCHEMICAL ANALYSIS OF SEVEN PLANTS BELONGING TO FAMILY CAESALPINIACEAE WITH SPECIAL EMPHASIS ON QUININE

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A B S T R A C T

Present paper reports the comparative phytochemical analysis of seven plants belonging to family Caesalpiniaceae i.e. *Bauhinia acuminate* L., *Cassia angustifolia* L., *Cassia fistula* L., *Cassia occidentalies* L., *Cassia tora* L., *Saraca indica* L. and *Tamarindus indica* L. with special reference to quinine. Plant leaves were air dried in shed for 3 to 4 days at room temperature and then it was kept in hot air oven at 40 degree for 30 min until all the water molecules evaporated and plant became well dried for grinding. Four type of solvent were used i.e double distilled water, Ethanol, methanol and n-butanol for preparation of extracts. Standard protocol was used for Phytochemical analysis.

Qualitative Phytochemical analysis showed the presence of Quinine, Protein, Carbohydrate, Phenol, Tannin, Flavonoids, Saponins, Glycosides, Steroid, Alkaloids, and Terpenoids

INTRODUCTION

Phytochemicals are secondary plant metabolites present naturally in plants considered as "man friendly medicines" cure diseases without causing any harm to human begins. Major bioactive Phytochemical constituent are Quinines, alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds. (Edeoga *et.al* 2005). For the treatment of Malaria quinine is widely used and quinine is very effective easily available, avoid economic burden, and free from storage. Generally plant obtained quinines have no side effect. A sixteen member committee of the ICAR (Indian Council of Agriculture Research) has reported that the actual number of malarial death in India on an average would be around 40,297 around 40 times higher than present estimation in 2013. (Hindustan Times August 29, 2014). Kolhan region of Jharkhand is dominated by tribes and back trodden people and they are mainly depend, upon folk medicine for their primary health care.

World Health Organization WHO (2014) report that due to malaria about 4.28 million people were died out of which 55% belonging to south East Asia region. In NVBDCP (National Vector Borne Diseases Control Programme) ; 2016) reported that the total number of death caused by this disease is 562 in India out of which 8% belonging to Jharkhand and in which 75% was from Kolhan region. Kolhan region comprises three districts, Viz East Singhbhum, West Singhbhum and Seraikela Kharsawan and the total population of this region is 48,56,109. (2011 censes) Present research work has been design to find out the qualitative Phytochemical analysis with special reference to quinine.

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MATERIAL AND METHODS

Collection of plant materials

All seven medicinal plants *i.e. Bauhinia acuminate* L., *Cassia angustifolia* L., *Cassia fistula* L., *Cassia occidentalies* L., *Cassia tora* L., *Saraca indica* L. and *Tamarindus indica* L. belongs to family Caesalpiniaceae and were collected from different areas of Kolhan regions. The plants were identified with the help of referenced books like "A Hand Book Of Medicinal plants" (Narayan *et.al.* 2003), "Botany of Bihar and Orissa" (Haines, 1921), "Indian medicinal plants" (Kirtikar and Basu; 1918), "The Treatise on Indian Medicinal Plants" (Chatterjee and Prkrashi; 2013).

Preparation of powders

For Biochemical analysis the plant materials were shade dried for 3 to 4 days at room temperature and then it was kept in hot air oven at 40^0C for 30 min until all the water molecules evaporated and plants became well dried for grinding. Plant materials were ground well by using mixer grinder (Bajaj easy) into fine powder and kept into airtight containers for future use.

Preparation of plant extracts

The plant extract was prepared according to methods adopted by (Ahmad and Beg 1998) & (Kassa *et.al.* 2014) Four types

of solvent were used *i.e.* Double distilled water (DDW), Ethanol, Methanol and n-Butanol.

Preparation of Double distilled water (DDW), Extract Solution: **-** 5g of leaf powdered was added in 100 ml of doubled distilled water in a conical flask and kept on magnetic stirrer for 30 min at room temperature and was left for 72 hours (Ahmad *et.al.* 1998) and then it was again kept on magnetic stirrer for 30 min and filtered with the help of whatman filter paper 125mm Cat no 1001 125 and filtrate was stored in refrigerator for further uses.

Preparation of Ethanolic Extract Solution**:** - 5g of leaf powdered was added in 100 ml of ethanol in a conical flask and kept on magnetic stirrer for 30 min at room temperature and was left for 72 hours and then it was again kept on magnetic stirrer for 30 min and filtered with the help of whatman filter paper 125mm Cat no 1001 125 and filtrate was stored in refrigerator for further uses.

Preparation of Methanolic Extract **-:** 5g of leaf powdered was added in 100 ml of methanol in a conical flask and kept on magnetic stirrer for 30 min at room temperature and was left for 72 hours and then it was again kept on magnetic stirrer for 30 min and filtered with the help of whatman filter paper 125mm Cat no 1001 125 and filtrate was stored in refrigerator for further uses.

Preparation of n- Butanolic Extract **-:** 5g of leaf powdered was added in 100 ml of n –butanol in a conical flask and kept on magnetic stirrer for 30 min at room temperature and was left for 72 hours and then it was again kept on magnetic stirrer for 30 min and filtered with the help of whatman filter paper 125mm Cat no 1001 125 and filtrate was stored in refrigerator for further uses.

Qualitative Phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods [Sofowra, 1993, Trease and Evans, 1989, Harborne, 1973].

Test for Quinines**: -** Four methods were adopted for detection of quinine

By concentrated H_2SO_4 method**: -** In 2ml of extract, 1 ml of concentrated sulphuric acid was added, development of red color shows the presence of Quinines.(Kalaiyarasan *et.al.* 2012), (Anitha *et.al.* 2013), (Gincy *et.al.* 2014) & (Kakad *et.al.* 2014).

By concentrated HCl method: - In 2ml of extract, a few drops of concentrated Hydrochloric acid was added, development of Yellowish brown color shows the presence of Quinine.(Karthiyayini and Nithiya 2015)

By 10% Sodium Hydroxide Solution (NaOH) method **: -** In 2ml of extract 2ml of 10% sodium hydroxide solution (NaOH) was added the appearance of blue, green or Red color showed the presence of Quinines.(Rameshkumar *et.al.* 2015) & (Kassa and Mesay 2014)

By 1% alcoholic Potassium Hydroxide Solution (KOH) method **: -** In 2ml of extract, a few drops of 1% alcoholic KOH was added the presence of red to blue color indicates the presence of Quinines.(Tensingh *et.al.* 2015), (Tensingh *et.al.* 2014), & (Tiwari *et.al.* 2015)

Test for proteins

Millon's ***Test Method*****: -** In 2ml of extract, 2ml of Millon's reagent was added; then white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin Test Method**;** - In 2ml of extract 2ml of 0.2% solution of Ninhydrin, was added and boiled for 3 to 5 min, the development of violet color shows the presence of amino acids and proteins.

Test for carbohydrates

Fehling's ***Test Method*****: -** Equal volume of Fehling A and Fehling B reagents were mixed together and it was added to 2ml of crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Table 1 Phytochemical Analysis of Bauhinia acuminate L., Cassia angustifolia L., Cassia fistula L., Cassia occidentalies L., Cassia tora L., Saraca indica L. and Tamarindus indica L.

S. No	Name of Plants	Tests	Bauhinia acuminate L.				Cassia angustifolia L				Cassia fistula L.				Cassia occidentalies L.				Cassia tora L.				Saraca indica L.				Tamarindus indica L.			
	Type of Sol →	T.No	D	E	M	N	D	E	M	N	D	E	M	N	D	E	M	N	D	E	M	N	D	E	M	N	D	E	M	N
1.	Quinine	A1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		A2	-	-	+	-	-	+	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		A3	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+	+	+
		A4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Protein	B1	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
		B2	+	+	-	-	+	+	-	+	-	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-
3.	Carbohydrate	C1	+	+	+	-	-	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
		C2	-	+	-	-	-	+	-	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
		C3	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
4.	Phenol & Tannin	D	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
5.	Flavonoids	E1	+	+	-	-	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	+	-	-	-	-	+	+	-
		E2	+	-	-	-	+	+	-	-	+	-	-	+	+	+	-	-	+	+	+	+	-	-	-	+	+	-	-	-
6.	Saponins	F	+	+	+	-	+	+	-	-	-	+	+	-	+	+	-	-	+	+	+	-	+	-	+	-	-	-	-	+
7.	Glycosides	G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
8.	Steroid	H1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	+	+	+
		H2	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+
9.	Alkaloids	I	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+
10.	Terpenoids	J	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	-	-	-	+	-	-	-	+	+	+	+

Benedict's ***Test Method*****:** -2ml of extract when added with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Iodine Test Method: **-** In 2ml of extract few drops of iodine solution was added, a dark blue or purple coloration indicated the presence of the carbohydrate.

Test for phenols and tannins

Ferric Chloride Test method**: -** In 2ml of extract 2ml of 2% solution of $FeCl_3$ was added. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoids

Shinoda Test Method**: -** In 2ml of extract few fragments of magnesium ribbon was added then concentrated HCl was added drop wise. Pink scarlet color appeared after few minutes indicated the presence of flavonoids.

Alkaline Reagent Test Method**: -** In 2ml of extract 2ml of 2% solution of NaOH was added, an intense yellow color was formed which turned colorless on addition of few drops of diluted sulphuric acid indicates the presence of flavonoids.

Test for saponins

Foam Test Method**: -**In 2ml of extract 5ml of distilled water was added and then it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Keller-kilani test method**:** - In 2ml of extract 2ml of glacial acetic acid (CH_2COOH) was added followed by 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 inter phase indicated the presence of cardiac glycosides.

Test for steroid

Chloroform Test method**: -** In 2ml of extract 2ml of chloroform was added and then 2 ml of concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

Acetic acid method**: -**In 2ml of extract 2ml of Acetic acid (CH_2COOH) was added and then1 ml of Concentrated H_2SO_4 was added drop wise, the presence of Blue Green color indicates the presence of Steroids.

Test for alkaloids

Mayer's and Wagner's reagents Test method: **-** In 2 ml of extract 2ml of 1% HCl acid was added and heated gently. Mayer's and Wagner's reagents were then added in equal proportion.. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for terpenoids

Chloroform Test Method**:** - 2ml of extract was dissolved in 2ml of chloroform and evaporated to dryness, then 2ml of concentrated H_2SO_4 was added sidewise and heated for few min. A grayish color indicated the presence of terpenoids.

RESULT AND DISCUSSION

Qualitative Phytochemical analysis of *Bauhinia acuminate* L., *Cassia angustifolia* L., *Cassia fistula* L., *Cassia occidentalies* L., *Cassia tora* L., *Saraca indica* L. and *Tamarindus indica* L. belonging to family Caesalpiniaceae, are presented in Table -1 Our result showed the presence of Quinine, Protein, Carbohydrate, Phenol, Tannin, Flavonoids, Saponins, Glycosides, Steroid, Alkaloids, and Terpenoids, in DDW, ethanol, methanol, and n-butanol. It was interesting to note that maximum Phytochemicals were present in Ethanolic extract solution where as minimum found in n- butanol extract solution. Quinine was present in all the sevent plants and also reported in *Saraca indica* L (Kakad *et.al*. 2014) and *Cassia angustifolia* L. (Singanaboina *et.al*. 2014.., Phytochemical are also useful for human beings such as antimicrobial activity shows by alkaloids, (Bonjean *et.al.* 1998), Flavonoids are also known as vitamin P or plant modifiers. (Veerachari *et.al.* 2011), Tannins reduce the risk of coronary heart diseases. (Janaky Ranjithkumar *et.al.* 2010) steroids are used as analgesic agents (Singh AP, 2006), Saponins are natural glycosides possessing cytotoxic activity. (Irma Podolak *et.al.* 2010).

CONCLUSION

By the result of qualitative phytochemical analysis it is clear that these plants may be use to cure Malaria because it contains quinine.

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