RESEARCH PAPER

Science



Study on Qualitative and Quantitative Estimation of Phytochemicals of Triphala Plants

KEYWORDS	Triphala Plants, phytochemicals, quantitave Estimation.									
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ABSTRACT World is endowed with a rich wealth of medicinal plants and Natural products of plants offer vast resource of newer medicinal agents with potential in clinical use. Extraction methods and solvent selections are important for the collection of the phytochemicals because all compounds are not dissolving in all extracts and those compounds quantity rates are also different in different solvents. For that which solvent obtain maximum phytochemicals is most important things and the present study was an attempt to analyze the phytochemical analysis of Triphala plants (Terminalia chebula Retz., Terminalia belerica Retz., Phyllanthus emblica L).

Introduction

The natural resources how so ever large are bound to diminish hence need effective strategy is needed for sustainable utilization. Cultivation of medicinal and aromatic plants is constrained due to lack of suitable technology, which led to low yield and poor quality. Consequently, medicinal herbs are predominantly harvested in sufficient quantities from the wild in an unregulated manner (Shabbir et al., 2003). There are many astonishing things to learn from the collector of these herbs. Triphala is the combination of three medicinal plants exteensively used in ayurveda from ancient time.

In trifala plants, one is Terminalia chebula Retz. (Harde), second one is Terminalia bellerica Retz. (Behada) and third one is Phyllanthus emblica L. (Ambla). T. chebula Retz, T. bellerica both the plant belong to family combretaceae and P.emblica L.belong to family Euphorbiaceae. T. chebula Retz. is used in many diseases laxative, diuretic, cardiotonic (Jagtap and Karkera, 1999) and also reported to be an antimicrobial (Sato et al., 1997; Ahmad and Aqil ,2007), hepatoprotective (Tasaduq et al., 2003; Tasaduq et al., 2006), anti- inflammatory (Srikumar et al., 2005), antioxidant (Lee et al., 2007) and adaptogenic (Rege et al., 1999). P. emblica has been used for the treatment of several disorders and the important constituent of plant leaves extracts also posses several pharmacological properties like anti-viral (HIV, AIDS, HERPES VIRUS, CMV) antimutegenic, anti-allergic, anti-bacterial activities (Khopde et al., 2000).P. emblicaLcontains different class of secondary metabolites (Calixto et al, 1998). T.bellerica is used in the treatment of fever, cough, asthma, urinary diseases, piles, chronic diarrhea, dysentery, flatulence, vomiting, colic and enlarged spleen and liver (Cheng et al., 2003).

The present study was carried out with an aim to estimate the amount of phenolic acids and flavonoids which are the constituents that may be responsible for the various pharmacological activities exhibited by the plan.

Methodology

Collection and preparation of Plant Extracts Materials

The Sample of triphala plants were collected from Botanical garden of Hemchandracharya North Gujara University. The leaf and stem of the plant samples were collected from botanical garden. Here extraction was prepared in maceration method. Collected leaves and stems are washed under tap water than cut in to small pieces and again washed in distill water Then leaves and stems were left for drying under shade and after air drying they were grinding. 1 gms of each sample was soaked in conical flask containing 20ml water, methanol, Petroleum ether, chloroform, ethyl acetate and acetone for 24hour. After filtered of extracts the supernatants were collected, covered, labeled, stored at 20° C and used for the screening of various phytochemical and quantative estimation (Prashant et al., 2011).

Qualitative and Quantitative estimation of phytochemical

Primary qualitative analysis was done by Talari et al., 2012 and Rajeshvari et al., 2011 methods. Total phenol was determined by Folin Ciocalteu reagent (McDonald et al., 2001) and Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Quantitative estimation was done by regression method. For Total Phenol gallic Acid was used as standard (Fig:1) and For Total Flavonoids quercetin was used as standard (Fig:2).

Result and Discussion

Qualitative Analysis

The result of gulitative analysis was shown in Table No; 1.Phytochemicals screening of Terminalia chebula Retz. alkaloids was detected in water, methanol, petroleum ether and acetone of leaves but for stem alkaloids was detected in methanol and in acetone extracts. In Terminalia bellerica Retz. that was showed positive result in water, methanol, ethyl acetate and acetone extracts of leaves and same result was observed in Phyllanthus emblica L but only for leaves. For stem extracts showed positive result in water and methanol extracts of T.bellerica Retz. and in Phyllanthus emblica L, showed same result like T.chebula Retz. Alkaloids test was obtained positive in water, methanol and acetone extracts for leaves of all the plants and same things observed positive in water and methanol but for stem. Among three plants, in two plants tannins were detected in water, methanol and acetone but in T.bellerica Retz.



Fig 1: Standard of Gallic acid for Total phenol.



Fig 2: Standard of Quercetin for Total Flavonoids.

Table 1; Qualitive	analisys	of phyt	ochemical	in	various	sol-
vents						

me olants	me Extracts		Alkaloid		Tannin		Glycoside		Flavonoid		Lignin		Quinone		Saponin		Sterol	
of	Za	q	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
	Wa		+	-	+	+	-	+	+	+	-	-	-	-	+	-	-	-
	Me		+	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
T.	P.E		+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
chebula Retz.	Ch		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	Et		-	-	+	-	+	+	-	-	-	-	-	-	-	-	+	+
	Ace		+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
	Wa		+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-
	Me		+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-
T. bellerica	P.E		-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Retz.	Ch		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Et		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ac		+	-	-	-	+	+	+	+	+	+	-	-	+	+	-	-
	Wa		+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
D	Me		+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-
l.	P.E		-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-
suidina	Ch		-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
L.	Et		+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
	Ac		+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-

Note: += Positive, -=Negative, Wa= water, Me= methanol, P.E= petroleum ether, Ch=chloroform, ET= ethyl acetate, Ac= acetone. 1=leaves, 2= stems.

That was detected in water and methanol extracts. In all extracts tannin were present in acetone extracts for three plants. Preliminary test of flavonoids detected in water, methanol and acetone extracts of all the plant parts. Glycosides were present in ethyl acetate and acetone extracts for leaves of Terminalia chebula Retz. and in stems that was detected in water, methanol, ethyl acetate and acetone extracts for stem. In plant parts of T.bellerica Retz. glycosides were detected in water and water and that was observed in one more extract that was methanol. In Phyllanthus emblica L. glycosides test showed positive result in all extracts. For leaves lignin was present only in Terminalia bellerica Retz. that in methanol and acetone extracts and in other two plants that was absent in all extracts. Lignin was found only in acetone extract for both plants and absent in p. emblica L. In stems at showed better result in compare to other extract in both plant parts but that lignin was absent in Terminalia chebula Retz. and Phyllanthus emblica L. Quinones test showed negative result in all extracts of all the plant parts. In T. chebula Retz. saponins were present in water, methanol and acetone extracts for leaves but for stem it was also absent in water both the plants parts T. bellerica Retz. that was present petroleum ether and acetone extract but P.emblica L. also a present was one more extract methanol. Plant parts of the T. chebula Retz. sterol was present in four extracts but absent in water and acetone extracts but in case of the other two plants sterols were absent in all extracts. In T. chebula Retz. alkaloids, flavonoids, tannins, glycosides, lignin, saponins and sterol were present in both plant parts in different extracts but quinines were absent in all extracts. Phytochemical analysis of T. chebula in methanolic extract showed the presence of flavonoids, polyurinoids, saponins, tannin and absence of alkaloids, steroids (Kumar et al., 2013). In T.bellerica Retz. alkaloids, flavonoids, tannins, glycosides and saponins were present of leaves in different extracts and lignin, sterol and quinines were absent in all extracts but for stem with components lignin was also showed positive result. That means lignin was present in only stem of T. bellerica Retz. Same result were reported that alkaloid, phenol flavonoids, triterpenoids, steroids, tannins, glycosides were present but quinones and saponins were absent in methanolic extracts (Mety and Method, 2011). Saponins showed positive result in petroleum ether extract not in methanol. So, if we collected saponins component the extraction was prepared in petroleum ether extract. The result of qualitative screening of phytochemicals components in leaves and stems of Pemblica L. revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and quinones, lignin and sterols were absent. Same type of work done by was supported by Dhale and Mogle, 2011 in P.emblica showed the presence of alkaloids, oil fat, glyceroids, carbohydrates, phenolic, tannins, lignin, saponins, flavonoids and terpenoids.

Table 2; Quantative estimation of Total Phenol and Flavonoids of Triphala plar	nts
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Sr. No	Name of Plants	Total Phenol in	mg/g/ml			Total Flavonoids in µg/g/ml					
		Leaves		Stems		Leaves		Stems			
		Me.	Ac.	Me.	Ac.	Me.	Ac.	Me.	Ac.		
		Extract	Extract	Extract	Extract	Extract	Extract	Extract	Extract		
1	T. Chebula Retz.	24.35± 0.17	11.85± 0.31	14.4± 0.43	3.15± 0.24	767.33± 0.33	576.67± 0.33	561.33± 0.66	300.33±0.33		
2	T. Bellerica Retz.	28.51± 0.13	2.05± 0.27	8.03± 0.21	0.9± 0.30	811.33± 0.88	737± 0.66	634± 2.08	325.33±0.66		
3	P. emblica L.	24.23± 0.66	2.93± 0.03	11.15± 0.23	1.8± 0.34	1267± 0.66	477± 0.66	509.67± 1.45	152± 1.00		

The data is represented in Mean±S.E for five replicates

Quantitave Estimation of Total Phenol and Total Flavonoids.

Over all total Phenol and total flavonoids content are higher in methanol extract compare to acetone extract. Total phenol is higher Terminalia bellerica Retz. among all the plants for leaves and for stem it is higher in Terminalia chebula Retz. Total flavonoid is higher in P.emblica L. among all the plants for leaves and for stem that was in T.bellerica Retz. in methanol extract but in acetone extract higher value was obtained in T.bellerica Retz. for both the plant parts (Table; 2). This type works was supported by Mety and Mathad, 2011 observed the phenol and flavonoids content were determined in all plant parts in both extract and higher content were observed in methanolic extracts compared to ethanolic extracts. Total phenol and total flavonoids content was higher in methanol extracts than acetone extracts and that was in methanol extract for leaves of Tecomella (Vaghasiya et al., 2011). Intention of this studied was to correlate relationship of these secondary metabolites to possible biological activities and evaluate as a potential source of natural bioactive chemicals

Conclusion

In Triphala plants, over all alkaloids, flavonoids, glycosides and tannins are more detected in methanol, water and acetone extracts. In Terminalia bellerica Retz Lignin are present in methanol and acetone extract of and absent in other two plants . Quinones are absent in all the plant and also in all extracts. For all the plant saponins are found in petroleum ether extract. Sterol is absent in all the plants. For collection of the total Phenol and flavonoids methanol extract is good.

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