The Journal of Phytopharmacology 2015; 4(3): 172-175
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(3): 172-175
May- June

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Preliminary phytochemical examination of *Homonoia riparia* Lour. and its effect on clotting time *in vitro*

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Abstract

Whole plant parts of *Homonoia riparia* Lour. (Euphorbiaceae) is claimed to be active against various ailments like constipation, emesis, piles, bladder stones, gonorrhoea, syphilis, toothache, angina, malaria, and wounds caused by scorpion and fish bites. It is claimed to have blood clotting property as leaves are used to stop bleeding in fresh cut wounds in folklore practice. The present study was carried out to evaluate the phytochemical composition and effect of its extracts on clotting time. Leaf sample of *H. riparia* was standardized for authenticity, quality and chemical composition using Pharmacopoeial procedures. Preliminary phytochemical tests were performed using procedures of phytochemical testing. Chloroform and ethanol extracts of air dried leaf at different dose levels were tested on clotting time on blood of healthy volunteers following Lee and White method. Physico-chemical constants and HPTLC fingerprint of the *H. riparia* leaf was recorded. Preliminary phytochemical tests revealed presence of alkaloids, carbohydrates/glycosides, carboxlic acids, flavonoids, phenols, steroids, saponins, tannins and terpenoids. The ethanolic extract at 100 mg was found to be better than chloroform extract. The activity is found to increase with increase in concentration. Standardized *H. riparia* investigated in the current study possess anticoagulant activity in contrast to the claim of blood clotting activity claimed in folk medicine.

Keywords: Folklore herbs, haemostatic, NSAIDs, phytochemical, prothrombin time.

Introduction

*Homonoiariperia*Lour. - Euphorbiaceae is a dioecious shrub growing to a height of 1.0 to 1.5 m in river banks with rocky boulders, often found in North, East and central India, the Deccan peninsula and Andaman Islands. It is found in in Karnataka and Maharashtra regions. Leaves alternate, glandular scaly beneath, flowers are small and unisexual in spikes; it bears capsular fruit with ovoid seeds [1].

Different parts of the plant are active against various ailments like roots for its laxative, diuretic and emetic property. Leaf juice regarded as depurative. The leaves are used to treat wounds caused by aquatic fish bites like scorpion fish [2, 3]. Pounded leaves and fruits are used as a poultice for skin diseases [3]. The leaves were used for cuts and wounds as per ethnobotanical reports [4]; it is used in piles [5]; for blood clotting and muscle fracture [6]; also for skin diseases [7]. Fifty per cent ethanolic extract of the plant (excluding root) was found to be inactive when tested for antibacterial, antifungal, antiprotozoal, antiviral and diuretic activities, and produced absolutely no effect on CVS, CNS and isolated tissues of experimental animals or in *ex-vivo* studies [8].

The leaves are reported to contain taraxerone and quercetin-3-O-β-D- glucopyranosyl [1-6]-O-α-L-rhamnoside as major chemical constituents [9].

The leaves are claimed to have anticoagulant property as it is used to stop bleeding in fresh cut wounds in folklore practice [6]. In the current study, leaves were characterised employing physico-chemical analysis, preliminary phytochemical examination and HPTLC. Chloroform and ethanol extracts of leaf of *H. riparia* was tested for coagulation time *in vitro.*

Material and methods

Collection of plant material and extraction

The plant was collected from Kabbinale, Hebri in Udupi district of Karnataka, it was authenticated by referring to flora of Udupi [10]. The plant material (Figure 1) (Voucher specimen number 606/14042901) was dried and powdered and used for preparation of extract. About 10 gm of the powder was loaded into a thimble of Soxhlet extractor and successively extracted with chloroform and ethanol.

Aerial parts Fresh leaves

Figure 1: *Homonoia riperia* Lour.

Standardization

The powdered plant material was standardized as per method [11].

Phytochemical screening

Total ethanol extract was tested for the presence of different phytoconstituents like alkaloid, steroid, flavonoid, tannin, glycoside etc [12].

HPTLC

Sample preparation

One gram of the powdered plant material which was previously dried and powdered was soaked in 10 ml alcohol for 24 hrs, filtered and filtrate was made up to 10 ml and used for application.

Development and documentation

Four and eight micro litres of the sample was applied on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (Merck, Germany) using CAMAG LINOMAT 5 applicator [13]. The plate was developed in CAMAG glass twin trough chamber previously saturated with mobile phase toluene: ethyl acetate (8.0: 1.0). The plate was derivatized using vanillin- sulphuric acid (VS), and heated at 105 °C till the spots appeared [14, 15]. The developed plates were visualized in CAMAG visualizing chamber and scanned in CAMAG Scanner 4 under 254, 366, 540 (pre-derivatisation) with the help of CAMAG WinCATS software. R_f values and densitograms were recorded.

Anticoagulant activity

Blood samples were obtained from 10 healthy volunteers, aged between 21-25 years. The subjects have been chosen for this study following criteria of having normal prothrombin time, not suffering from any cardiovascular diseases (hypertension, congestive heart failure, coagulation disorders such as, Hemophilia A or B) or diabetes, not recently using nonsteroidal anti inflammatory drugs, non obese or non smokers and free from dyslipidemic disorders. The blood samples were withdrawn from vein of right arm of each subject using sterile syringes, and placed separately in containers containing tri-sodium citrate to prevent the clotting process. This sample was further used for whole blood coagulation (clotting) time) with slight modification [16].

Results and discussion

Some known anticoagulant NSAIDs are heparin, dicoumarol, 4-hydroxycoumarin and warfarin. Adulthood cardiovascular diseases due to sedentary life style of urban people and lack of exercise leads to hypertension, cerebral hemorrhage, coronary thrombosis, arteriosclerosis and congestive heart failure. A number of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin have been used as antithrombotic agents. These drugs *in vitro* and *in vivo* cause inhibition of platelet aggregation and thromboxane formation[17-19]. Some of the dietary supplement which has been reported to have anticoagulant activity are onion, garlic, clove, fenugreek, mug wort, sage and marine algae [20-22]. Some potentiate the activity of warfarin and they increase prothrombin time and these can be used as adjuncts in preventive cardiovascular therapy, one such herb is Chinese herb Danshen [23].

Standardization was carried out as it is an important aspect in maintaining and assessing quality and safety of the crude drug. The herb was found to have no other parts as foreign matter. Only leaves of the sample was selected for the study. Loss on drying reveals the moisture content, the sample has 6.36% of moisture; total ash is the indication of total inorganic content, 8.68% ash was detected in the sample; acid insoluble ash is the acid insoluble part of total ash, mainly silica, the sample showed 0.2% acid insoluble ash; water soluble ash is the water soluble part of total ash indicating inorganic content without water insoluble inorganic salts like silica, 2.7% was water soluble; water and alcohol soluble extractive is indicative of percentage active constituents soluble in water and ethanol, the values were 15.94 and 11.14% respectively. These physico – chemical standards would indicate the purity and authenticity of the leaves of *H. riparia* (Table 1).

Table 1: Physico-chemical constants of *Homonoia riperia* Lour

Parameter	Mean±SEM
Foreign matter	Nil
Loss on drying at 105^0C	6.36 ± 0.001
Total ash	8.68 ± 0.150
Acid insoluble ash	0.20 ± 0.002
Water soluble ash	2.70 ± 0.005
Water soluble extractive	15.94 ± 0.135
Alcohol soluble extractive	11.14 ± 0.042

Results expressed as % w/w (n=3)

Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates/glycosides, carboxlic acids, flavonoids, phenols, steroids, saponins, tannins and terpenoids. The phytochemical constituents present in the extract can be held responsible for different medicinal activities of the plant (Table 2).

Table 2: Phytochemical screening of leaf extract of *Hoia romoniperia* Lour

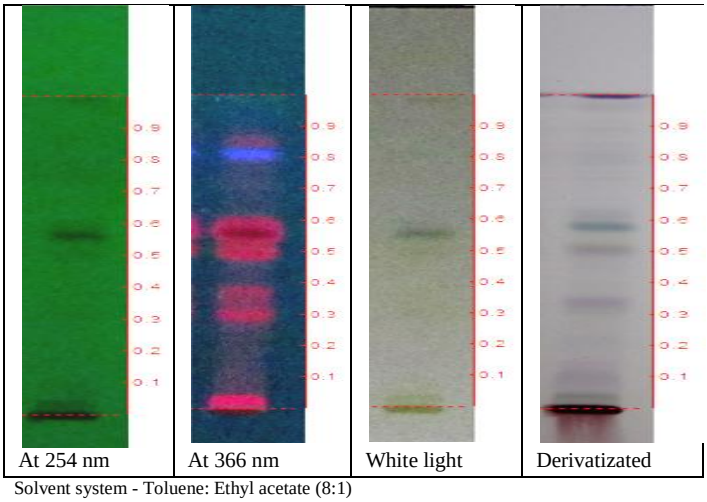
Phytoconstituents	Results
Alkaloids	+
Carbohydrate	+
Carboxylic acid	+
Coumarins	-
Flavanoids	+
Phenol	+
Quinone	-
Resins	-
Steroid	+
Saponins	+
Tannin	+
Terpenoid	+

HPTLC fingerprinting profile of the plant when scanned under 254 nm showed the presence of 2 spots (all in green) at R_f of 0.51 and

0.57, under 366 nm there were 6 prominent spots (Fluoroscent) at R_f of 0.30, 0.38, 0.51, 0.57, 0.82, 0.86 and when scanned under white light at 540nm spots were present at R_f 0.51 and 0.57, following post derivatisation with vanillin sulphuric acid spots(in different colors) were evident at R_f 0.05, 0.11, 0.15, 0.23, 0.35, 0.51, 0.59, 0.64, 0.80. Among these the spots were common at R_f of 0.51 and 0.57 (except at postderivatisation) at different color intensities (Table 3 & Figure 2).

Table 3: Effect of extract of *Homonoia riperia* on blood clotting time

Extract	Concentration (mg)	Clotting time (Mins ± SD)
Control	-	3.30 ± 0.29
Chloroform	50	6.00 ± 2.15
	100	12.00 ± 3.58
Ethanol	50	12.32 ± 3.25
	100	17.76 ± 2.58



Solvent system - Toluene: Ethyl acetate (8:1)

Figure 2: TLC photodocumentation of total ethanolic extract of *Homonoia riperia* (8 µl)

R_f values by densitometric scan of *Homonoia riperia* Lour. showed 10 spots at 254nm, 9 spots at 366nm, and 5 spots at 540nm respectively (Table 4 & Figure 3).

Table 4: Rf values by densitometric scan of *Homonoia riperia*

R_f values	Peaks at 254 nm	Peaks at 366 nm	Peaks at 540 nm
0.02	41.02	23.64	30.76
0.06	5.18	6.21	4.53
0.13	2.02	2.64	-
0.37	3.71	8.92	1.74
0.45	1.83	8.42	-
0.51	1.00	-	-
0.59	5.92	8.14	9.86
0.66	26.013	40.97	53.12
0.78	0.86	-	-
0.87	12.32	-	-
0.92	-	0.60	-
0.98	-	0.45	-
Total	**10**	**9**	**5**

Values in % area; highlighted values are compounds with same R_f

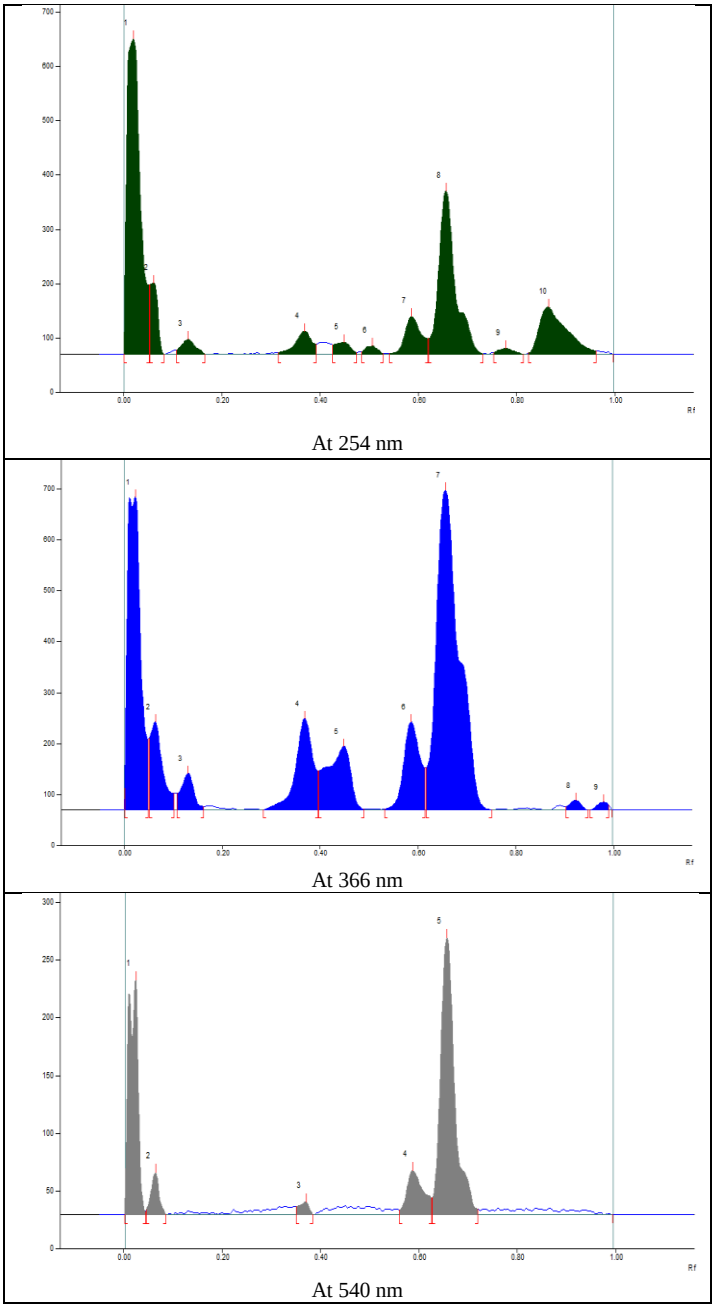


Figure 3: Densitometric scan *Homonoia riperia* (8µl)

Anticoagulant activity of chloroform and ethanol extract was assayed comparatively. The anticoagulant activity was good at concentration of 100 mg in both the extracts when compared to 50 mg. The ethanolic extract was proved to be better than chloroform extract (Table 4). Clotting of blood involves in two pathways: the extrinsic and intrinsic pathways [24]. The extrinsic pathway usually produces clot in as little as 15 s, while the intrinsic pathway requires 2-6 min [25], so anticlotting effect of *H. riperia* may be attributed to intrinsic pathway. Negative charged polyphenolicpolysaccharides were suggested to have played a role in the anticoagulant activity, especially prolonging blood coagulation in the intrinsic pathway [26]. Phenolics together with carbohydrates may also have pivotal role to play in anticoagulant activities [27-298]. Flavonoids prevent platelet aggregation and thereby reduce the risk of cardio vascular diseases [30]. Polyphenolic polysaccharides, phelols and flavonoids can be held responsible for the possible anticoagulant activity in *H. riperia.*

Conclusion

An anticoagulant with better safety margins and with no monitoring of therapy is of utmost importance for laboratory and clinical use.

Anticoagulant activity was dose dependent in both chloroform and ethanolic extracts. There is a need to take it further for *in-vivo* anti-coagulant activity in clinical trial subjects. An investigation into the action of single bioactive compound in blood coagulation pathway can be carried out in the isolated fraction which can be undertaken further in future.

Acknowledgement

Authors are grateful to revered President, Dr. D. Veerendra Heggade, SDM Educational Society for the encouragement.

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