Indian Journal of Natural Products and Resources
Vol. 8(3), September 2017, pp. 248-253

Evaluation of *in vitro* antimicrobial activity, qualitative and quantitative phytochemical, proximate composition of leaf and stem of *Reinwardtia indica* Dumort: A comparative study

Abha Shukla[1*], Swati Vats[1], Rishi Kumar Shukla[2], Deepak Painuly[2], Anirudh Porval[2] and Jashbir singh[2]

[1]Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar249407, Uttarakhand, India

[2]Department of Chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar249404, Uttarakhand, India

Received 25 March 2015; Revised 12 June 2017

Reinwardtia indica Dumort as evaluated for its antimicrobial potential, qualitative and quantitative estimation of phytochemicals, and proximate composition analysis by using standard methods. The antimicrobial activity was evaluated by the agar well diffusion method against four bacterial strains and two fungal strains. Ethanol and chloroform extract of the leaf and the stem are highly active against all the strains. This might be due to the presence of different phytochemicals such as alkaloids, flavonoids, glycosides. The result of the proximate composition showed that the stem contained higher values of crude proteins, crude lipids, carbohydrates, and nutritive energy. The stem bark was richer in carbohydrates than the leaves. From the results, it can be concluded that *R. Indica* may be a good source of energy, is rich in phytochemicals, and have antimicrobial nature, thus, may be useful in various pharmaceutical formulations.

Keywords: Antimicrobial activity, Nutritive Value, Phytochemical, Proximate Composition, *Reinwardtia indica* Dumort

IPC code; Int. cl. (2015.01)−A61K 36/100

Introduction

India is well known for its traditional knowledge of herbal medicine as mentioned in *Ayurveda*. In *Ayurveda*, numerous plants have been designated as medicinal plants[1]. Since that time, plant products that are derived from plant parts such as stem, bark, leaves, fruits, and seeds have been part of phytomedicine[2]. In addition, the use of herbal medicine for treatment of disease is as old as mankind and now, World Health Organisation (WHO) also supports the use of traditional medicine provided they are proven to be efficacious and safe[3]. Therapeutic properties of herbal drugs are mainly due to the presence of some natural bioactive compounds commonly known as phytochemicals[4].

In spite of tremendous progress in human medicine, infectious disease caused by bacteria, fungi, viruses, and parasites are the major threat to public health and are responsible for about half of the death in tropical countries[5]. A natural product from higher plants may possess a new source of antimicrobial agent with possibly a new mechanism of action[6]. They are effective in the treatment of infectious diseases with simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[7].

On taking account of the importance of natural herbs as potent antimicrobial agent, the present study was based on *in vitro* antimicrobial activity and its correlation with the phytochemical present in *Reinwardtia indica* Dumort.

R. indica belongs to Linaceae family, which is also known as flax family. *R. indica* comes from foothills of Himalaya. Its common name is linum or yellow flax having a small evergreen shrub growing to about 1m tall. It forms bushy clump with erect stems sucking from the base. The oval shaped leaves are lime green in colour. In winters it produces masses of five petaled butter yellow flower shown in the Plate 1. The plant is widely used by local communities for different medicinal purposes like for tongue wash, for increase in lactation period, in wound infection, and against skin diseases etc[8].

The present study was designed to evaluate the stem extracts of *R. Indica* for qualitative and quantitative phytochemical, proximate content and also to compare antimicrobial activity between leaves and stems.

*Correspondent author
Email: abs.gkv@gmail.com

Plate1 — Flowers and leaves of *Reinwardtia indica* Dumort

Materials and Methods

Plant material

Fresh leaves and stems of *R. indica* were collected from Forest Research Institute, Dehradun, Uttarakhand, India. Plant material was authenticated by Botanical Survey of India, Dehradun. A herbarium (accession no- 114094) was deposited in the Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar (India) for future reference.

The fresh leaves and stems were washed with water and then dried under the shade for 15 and 30 days, respectively. The dried leaves and the stems were powdered separately by crushing in the grinder and then stored in air tight container for extraction and other experiments.

Proximate composition

Proximate analysis of the powdered stems includes estimation of moisture content, ash content, crude fibre, crude fat, protein content[9], whereas total carbohydrate were calculated by the following equation[10]:

Total carbohydrate = 100 - (% Ash+ % Moisture + % Crude fibre+ % Crude protein)

Nutritive value

Nutritive value of stems was expressed in Kilocalories/ 100g of dry weight of leaves and stems. Nutritive value was calculated by using the given formula[10]:

Nutritive value = (4× % Protein) + (9× % Crude fat) + (4× % Total carbohydrate)

Quantitative Phytochemicals

Defattening of dried sample

Weighed the dried stem and extracted it with petroleum ether or diethyl ether with the help of soxhlet. The Soxhlet was run for about 72 h or till the solvent coming out of the siphoning tube became colourless[11].

Estimation of alkaloids

About 5 g of defattened stem sample (W_1) was taken in 250 mL beaker, to which 200 mL of 10 % acetic acid in ethanol was added and kept for 4 h. It was then filtered through whatmann filter paper no. 42. The solution was concentrated in a water bath till about one fourth of the original volume was left. Then, concentrated NH_4OH was added drop wise, till the precipitate was formed. Precipitate was collected, washed with NH_4OH, and filtered. The residue was dried and weighed (w_2). The W_2 is alkaloid content in mg/g of dry weight of sample[12].

Estimation of flavonoids (gravimetric method)

About 5 g of defattened stem sample was taken and boiled in 50 mL of 2M HCl for 30 min under reflux. It was allowed to cool and then, it filtered through Whatmann filter paper no. 42. A measured volume of extract was fractioned with equal volume of ethyl acetate in separating funnel. This process was repeated for three times. The ethyl acetate layer consists of flavonoids. Dried the ethyl acetate layer and weighed it[12].

Estimation of saponins

About 20 g of defattened stem sample was put in a conical flask, to which 100 mL of 20 % aqueous ethanol was added. The samples were heated on a water bath for 4 h with continuous stirring at about 55 ºC. The mixture was filtered and the residue was re-extracted with another 200 mL of 20 % ethanol. The combined extracts were reduced to 40 mL over water bath at about 90 ºC. The concentrate was transferred into a 250 mL separating funnel, to which 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and ether layer was discarded. The purification process was repeated. Then, added 60 mL of n-butanol and washed the extract with 10 mL of 5 % aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight and saponin content was calculated[12].

Estimation of glycosides

The quantity of cardiac glycosides in the raw and treated samples was evaluated using Baljet's reagent. About 1 g of each defattened stem sample was soaked in 10 mL of 70 % alcohol for 2 h and then filtered. The extracts obtained were then purified using lead acetate

and disodium hydrogen phosphate solution before adding freshly prepared Baljet's reagent. The intensity (absorbance) of the colour produced was then measured using a spectrophotometer at 495 nm. The difference between the intensity of the colour of the experimental and blank (distilled water and Baljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycoside[13].

Preparation of extracts

About 150 g of the dried powdered leaves and stems of *R. indica* were weighed, loaded, and extracted by Soxhlet apparatus using 1.5 L each of petroleum ether (PE), chloroform (CL), ethanol (ET), and water (AQ), respectively in accordance of hierarchy of polarity of solvents separately. Extraction was continued for about 72 h or till the solvent coming out of the siphoning tube become colourless. Extracts were concentrated under reduced pressure in rotary vacuum evaporator and refrigerated for further use.

Qualitative phytochemicals

Phytochemical analysis for various phytochemicals of the extracts was undertaken using standard qualitative methods[14]. The extracts were analysed for the presence of biologically active compounds like alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids, fat and oil, saponins, protein, etc.

Antimicrobial activity

Microorganisms tested

The bacterial and fungal strains used to assess the antimicrobial properties of different solvent extract of *R. indica* included two gram-positive bacteria *Bacillus cerus* (MTCC-430), *Staphylococcus aureus* (MTCC-737), two gram-negative bacteria *Pseudomonas aeruginosa* (MTCC-1688), *Salmonella entrica* (MTCC-98) and two fungal strains, filamentous *Aspergillus flavus* (MTCC-227) and non-filamentous *Candida albicans* (MTCC-277).The tested bacterial and fungal strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh, India in lyophilized form. The organisms were first grown on Muller Hinton broth and Sabourd dextrose broth respectively for bacteria and fungi. Then, they were cultured in agar medium before use. The microorganisms studied are clinically important ones causing several infections, food borne diseases, skin infections.

Antimicrobial assay

In vitro antimicrobial activity of the different solvent extracts of *R. indica* was studied against six strains by agar well diffusion method[15]. For the better assumption *in vitro* anti-microbial activity of leaf and stem of *R. Indica* were compared. Muller Hinton agar and Sabouraud dextrose agar were used for antibacterial and antifungal activities, respectively. The extracts were diluted in 100 % dimethyl sulfoxide (DMSO) at a concentration of 50 mg/mL. The agar was melted and cooled to 45 ^{o}C and a standardized inoculum (10^5 to 10^8 CFU/mL) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. Test compound (40 µL) was introduced in the well (6 mm). The plates were incubated over night at 37 ^{o}C for 24 h and 28 ^{o}C for 48 h of bacteria and fungi, respectively. Ofloxacin was used as standard drug for bacterial strains and fluconazole was used as standard drug for fungal strains. The well devoid of extract but with DMSO served as control. The activity was evaluated by measuring the zone of inhibition (in mm) against the test organisms. All the test process was performed in triplicate in laminar chamber.

Statistical analysis

The experimental results are expressed as mean±standard deviation of triplicate measurement and these are processed using Microsoft Excel 2010.

Results and Discussion

Results of proximate analysis of stem of *R. indica* are shown in Table1. The proximate analysis revealed that moisture content in stem is low, which shows that the material have long shelf life being less active against growth of microorganisms as moisture content is within the limit of 5-15 %[16]. Ash content is high in stem, which indicates that it is rich in mineral elements. Stem is a good source of energy as it has high fibre

Table 1 — Proximate content of stem of *Reinwardtiaindica*

S. No.	Parameter	Stem
1	Moisture content	7.8±0.07
2	Ash content	7.64±0.04
3	Crude fibre	0.449±0.03
4	Crude fat	2.03±0.30
5	Total protein	7.07±0.12
6	Total carbohydrate	82.82
7	Available Carbohydrate	82.36
8	Nutritive value	377.794*

*nutritive value is calculated in Kcal/100 gm dry weight of leaf & stem and all other parameters are in percentage

Values are expressed as mean ± standard deviation of the three replicates

content. Stem has low amount of fat so it is not harmful as a dietry supplement. Protein is in good amount in stem as it helps formation of bones, hairs, antibodies etc. Stem is also rich in carbohydrate that is essential for maintenance of life in plant and animals[18]. Nutritive value is expressed in terms of kcal/100 g dry weight of material. Overall, stem of *R. indica* shows good proximate content and nutritive value so can be act as dietary supplement along with food.

Table 2 gives the result of the quantitative phytochemicals. Stems showed the presence of rich amount of different phytochemicals. Phytochemicals may exhibit antimicrobial activity by different mechanisms for e.g. flavonoids have the ability to form complex with extracellular and soluble proteins and thus, destroy cell wall of the strains[19]. Antimicrobial susceptibility of saponins may be attributed to the tendency of causing leakage of protein and certain enzymes from the cell wall[20]. Steroids have been reported to have antibacterial properties. It has been reported to affect major detoxifying enzymes[21].

Results of the qualitative phytochemicals are given in Table 3. The results show that stem extracts are rich in alkaloids, which are the major class of phytochemicals that can act as painkiller, antibacterial etc[22]. Other than alkaloids, stem extracts equally showed the presence of flavonoids, steroids, glycosides, tannins, terpenoids, carbohydrates, saponins etc. Out of these phytochemicals, tannins and flavonoids are mainly known for their antioxidant properties[23]. Glycosides are usually active against various heart diseases by inhibiting sodium and potassium ion pump and increase the availability of sodium and calcium ions to heart muscle thereby improving cardiac output[24]. Saponins are bioactive antibacterial agents; steroids have antimicrobials activities and cardio tonic activities etc[25].

Results of the antibacterial and antifungal activity are shown in Table 4 and 5. Both, the leaf and the stem showed good antimicrobial activity. Chloroform extract of the leaves and stem exhibited activity against

Table 2 — Quantitative estimation of phytochemical from dry stem.

S. No.	Phytochemical	Stem (mg/g of dried material)
1	Alkaloids	145.22±0.07
2	Flavonoids	16.32±0.31
3	Saponins	10.44±0.10
4	Glycosides (cardiac)	50.00±0.90

Values are expressed as mean±standard deviation of the three replicates

Table 3 — Qualitative phytochemical screening of *R. indica* stem extract

S. No.	Phytochemicals	Test performed	Stem extracts			
			PE	CL	ET	AQ
1	Alkaloids					
		Wagner's test	-	-	+	-
		Hager's test	-	-	+	-
		Dragendroff's test	-	-	-	-
		Mayer's test	-	-	+	-
2	Flavonoids					
		Alkaline test	-	-	+	+
		Lead acetate test	-	-	+	+
3	Carbohydrates					
		Molisch's test	-	-	+	+
		Bendict's test	-	-	+	+
4	Tannins					
		Ferric chloride test	-	+	+	+
		Gelatin test	-	+	+	+
5	Glycosides					
		Legals test	+	+	+	+
		Keller Killiani test	-	+	-	-
		Born tragers test	+	+	-	-
6	Terpenoids					
		Liebermann burchard test	+	-	-	-
		Salwoski test	+	+	-	-
		Salwoski test (Triterpenes)	+	+	-	-
7	Steroids					
		Liebermann burchard test	+	+	-	-
8	Fat and Oil					
		Saponification test	+	+	-	-
		Filter paper test	+	+	-	-
9	Saponin					
		Foam test	-	-	+	+
		Froth test	-	-	+	+

+ = present, - = absent,
PE=Petroleum ether, CL= Chloroform, ET= Ethanol, AQ= Aqueous

Table 4 — Antibacterial activity of *Reinwardtia indica* leaf and stem extracts (50 mg/mL)

Plants	Zone of Inhibition (in mm)			
	S. aureus	*B. cerus*	*P. aeruginosa*	*S. entric*
Leaf				
PE	11.8±0.15	11.5±0.61	-	-
CL	9.1±0.27	9.5±0.50	-	15.2±0.91
ET	9.2±0.91	–	-	9.8±0.38
AQ	11.3±0.3	–	-	-
Stem				
PE	8.7±0.30	-	11.9±0.15	-
CL	10.7±0.30	11.9±0.45	9.9±0.41	-
ET	7.6±0.32	-	-	-
AQ	-	-	-	-
Ofloxacin	42.0±2.0	56.4±0.45	48.97±1.02	44.8±0.43

Values are expressed as mean ± standard deviation of the three replicates

Table 5 — Antifungal activity of *Reinwardtia indica* leaf and stem extracts (50 mg/mL)

Plants	*Zone of Inhibition in millimetre*	
	C. albicans	*A. flavus*
Leaf		
PE	-	15.2±0.31
CL	10.1±0.21	12.5±0.45
ET	11.4±0.13	12.4±0.15
AQ	-	14.2±0.10
Stem		
PE	12.3±0.36	11.2±0.20
CL	12.2±0.30	10.4±0.42
ET	10.4±0.21	10.5±0.10
AQ	-	11.5±0.09
Fluconazole	42.1±0.15	42.7±0.41

Values are expressed as mean ± standard deviation of the three replicates

all bacterial and fungal strain except *P. Aeruginosa* for leaf and *S. entric* for stem. This shows that chloroform extracts are selectively active against gram-negative bacteria and completely active against gram-positive bacteria. Whereas ethanol extract of the leaves was found to be active towards all the bacterial and the fungal strains except *B. cerus* and *P. aeruginosa*, while ethanol extract of the stem showed inhibitory activity against all the strains except *S. entric*. Petroleum ether and aqueous extracts of the leaf and the stem are active against only few bacterial strains but are highly active for both the fungal strains.

Conclusion

From the present study, it can be concluded that *R. Indica* stems as well as leaves have potent antimicrobial activity. Overall, the whole plant was found to have a vast range of phytochemicals and proximate content as well as nutritive value that makes this plant compatible for feed and fodder. Phytochemicals present in *R. indica* also give medicinal importance to it. Further identification of different phytochemical as active principle, *in vivo* antimicrobial activity, and different antimicrobial mechanisms are warranted.

References

1 Chandra P K, Medicinal plants conservation and enterprise development, *Med plants*, 2009, **1**(2), 79-95.

2 1soladoye M O and Chukwuma E C, Quantitative phyto–chemical profile of the leaves of *Cissus populnea* guill. & perr. (Vitaceae) – An important medicinal plant in central Nigeria, *Arc Appl Sci Res*, 2012, **4**(1), 200-206.

3 Hammuel C, Yebpella G G, Shallangwa G A, Magomya A M and Agbaji A S, Phytochemical and antimicrobial screening of methanol and aqueous extracts of *Agave sisalana*, *Acta Pol Pharma - Drug Res*, 2011, **68**(4), 535-539.

4 Amari N O, Bouzouina M, Berkani A and Lotmani B, Phytochemical screening and antioxidant capacity of the aerial parts of *Thymelaea hirsuta* L., *Asian Pac J Trop Dis*, 2014, **4**(2), 104–109.

5 Ofokansi K C, Attama A A, Uzor P F and Ovri M O, Evaluation of the combined antimicrobial activity of the leaf extract of *Phyllantus muellerianus* with ciprofloxacin, *J Pharma Tech Drug Res*, 2013, **2**(16), 1-6.

6 Hidayathulla S, Keshava C K and Chandrashekhar K R, Phytochemical evaluation and antibacterial activity of *Pterospermum diversifolium* blume, *Int J Pharm Pharm Sci*, 2011, **3**(2), 165-167.

7 Selvakumar S and Karunakaran C M, Antimicrobial efficacy of *Senna auriculata*, *Pongamia glabra* and *Indigo feratinctoria* against pathogenic microorganisms, *Int J Pharm Tech Res*, 2010, **2**(3), 2054-2059.

8 Shukla A, Vats S and Shukla R K, Preliminary phytochemical screening, antibacterial and nitric oxide radical scavenging activities of *Reinwardtia indica* leaves extract, *Int J Pharm Tech Res*, 2013, **5**(4), 1670-1678

9 AOAC, *The official method of analysis*, 15th edn, (Association of official analytical chemists, Washington D C), 1990.

10 Shukla R K, Painuly D, Porval A and Shukla A, Proximate analysis, nutritive value, total phenolic content and antioxidant activity of *Litchi chinensis* Sonn, *Nat prod Indian J*, 2012, **8**, 361-9.

11 Ganie A S, Agnihotri R K and Sharma R, Evaluation of *Duranta repens* for its antifungal potential, *Int J Med plants*, 2014, **106**, 390-395.

12 Harborne J B, *Phytochemical methods a guide to modern techniques of plant analysis*, 4th edn, (Springer), 1998.

13 El-olemy M M, Al-muhtadi F J and Afifi F A, *Experimental phytochemistry: A laboratory manual*, (King Saud University Press, Saudi Arabia), 1994, 21-27.

14 Evans W C, *Trease and Evans' Pharmacognosy*, 16th edn, (Elsevier Health Sciences, UK), 2009.

15 Bauer A W, Kirby W B, Sherris J C and Turk M, Antibiotic susceptibility testing by a standardized single disk method, *Am J Clin Pathol*, 1966, **45**, 493- 496.

16 Shukla R K, Painuly D, Shukla A, Kumar V, Singh J, Porval A, *et al*, Physical evaluation, proximate analysis and antimicrobial activity of *Morus nigra* seeds original article, *Int J Pharm Pharm Sci*, 2015, **7**(1), 191-197.

17 Oko A O, Ekigbo J C, Idenyi J N and Ehihia I U, Nutritional and phytochemical compositions of the leaves of *Mucuna poggei*, *J Biol life Sci*, 2012, **3**(1), 232-242.

18 Shukla A, Vats S and Shukla R K, Proximate composition, nutritive value and evaluation of antioxidant potential of stem of *Dracaena reflexa* Lam., *Int J Pharm Pharm Sci*, 2014, **6**(11), 360-364.

19 Pinho E, Ferreira I C F R, Barros L, Carvalho A M, Soares G and Henriques M, Antibacterial potential of North Eastern Portugal wild plant extracts and respective phenolic compounds, *Bio Med Res Int*, 2014, **2014**, 1-8.

20 Zablotowicz R M, Hoagland R E and Wagner S C, Effect of saponins on the growth and activity of rhizosphere bacteria, *Adv Exp Med Biol*, 1996, **405**, 83-95.

21 Epand R F, Savage P B and Epand R M, Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds, *Biochim Biophys Acta - Biomem*, 2007, **1768**(10), 2500-2509

22 Nagalingam S, Sasikumar C S and Cherian K M, Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit, *Asian J Pharm Clin Res*, 2012, **5**, 179-81

23 Shukla R K, Painuly D, Shukla A, Singh J, Porval A and Vats S, *In vitro* biological activity and total phenolic content of *Morus nigra* seeds, *J Chem Pharm Res*, 2014, **6**(11), 200-210.

24 Malu S P, Obochi G O, Edem C A and Nyong B E, Effect of methods of extraction on phytochemical constituents and antibacterial properties of *Tetracarpidium conophorum* seeds. *Global J Pure App Sci*, 2009, **15**, 373-376.

25 Mohanta T K, Patra J K, Rath S K, Pal D K and Thatoi J H, Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L. f, *Sci Res Essay*, 2007, **2**(11), 486-490.