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acta SATECH **1**(2): 133-137 (2004)

Research

Population variation in relation to phytochemical analysis of extracts in *Ipomoea triloba* L. (Convolvulaceae)

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Received: 9 Sept., 2003 *Revision accepted: 12 Dec., 2004*

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Abstract

18 populations of Ipomoea triloba sampled were classified into four categories of observable morphological differences. Chloroform extract of populations of two of these categories (Groups I & II) exhibited potent chemical activity resulting in the contraction of rabbit intestinal muscles. Concomitantly, the two population groups also possess high concentration of Tannins and Phenol. Tannin was highest in population groups Ia and II (0.56) followed by III (0.42) and lowest in IVb (0.29) mgml^{-1}. The populations contained fairly high phenol content (6.06-7.52) except group III which exhibited average values (3.55) mgml^{-1}. Populations Ia, II and IVa lacked saponin. Infrared Spectroscopy (IRS) phenol peaks (2925.1-2940.9cm^{-1}) were observed for all populations except group IVb. The differential chemical activity of populations of I. triloba confirmed its complex variable nature and useful taxonomically for intraspecific classification and identification.

Keywords: Chemical extracts, population variation, *Ipomoea triloba*, haemorrhoid, phenol

Introduction

Ipomoea triloba L. (Convolvulaceae) is a variable weed species of roadside, waste places, slow running water and riverbanks. Though of tropical American origin, the species is now circumtropical (Baker And Rendle, 1905; Van Ooststroom, 1953, Heine, 1963). Owing to its morphological variability, three varieties (*genuina* (α), *glaberrima* (β) and *eustachiana* (γ)) have been described in South and Central America (Meisner, 1869; Eggers, 1879). Two of these varieties (*genuina* (α), *triloba* (=*glaberrima* (β)) are recognised in Nigerian but quite variable with many intermediates.

Burkil (1966) reported that this species was used for headache. Ethnobotanical surveys also revealed that local traditional medicine practitioner use the plant for treatment of piles and haemorrhoids.

Haemorrhoids may be internal or external and result from abnormality of associated vessels, supporting tissues, mucous membrane or skin in the anal region (Mangan, 1988). Whereas genetic factors may not be ruled out, increased pressure or obstruction of various vessels in the anal region are important provoking factors. These include constipation, diarrhoea, abuse of laxatives, chronic coughing, severe liver disease, vomiting, sneezing, lifting of heavy goods, overweight, long standing or sitting, pregnancy and labour (White & Duncan, 1997).

Haemorrhoids are caused when cushion of blood vessels and bowel lining tissue in the rectal area swell above the internal spincter (internal haemorrhoid) or outside the external spincter (external haemorrhoid). Associated symptoms include itching, pain, bleeding, soreness, pruritis, burning sensation with passing stools, and inflammation. Severe pain accompanies external haemorrhoids due to the inflammation and edema from thrombosis whi-

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ch in turn may cause ischemia of the area and finally necrosis. Usually internal haemorrhoids are not painful until they bleed, prolapsed and enlarge. The chances of developing haemorrhoids increase with age as an estimated 50% of people at 50years and above have them at some time and to some extent (Smeltzer & Bare, 2000).

The study analyses the phytochemical components of the highly variable populations of *I. triloba*, the biological activity of its extracts and the taxonomic implications.

Material and methods

Field sampling

Populations of *I. triloba* were sampled between Ilishan-Remo and Abeokuta, Ogun State, Nigeria (c.70km stretch) to include possible variations found in herbaria, literature and the field. 18 populations divided into four groups of observable morphological differences, were examined.

Herbaria consulted included Forest Research Herbarium, Ibadan (FHI), University of Ibadan Herbarium (UIH), University of Lagos Herbarium (LUH), and Obafemi Awolowo University Herbarium (IFE).

Extraction

Plant shoots were air dried at room temperature ($26\pm1^{o}C$), ground, weighed and divided into eight portions in $250cm^3$-conical flasks. 20ml of Chloroform was added to the first four portions while 20ml of water was added to the rest and steamed at $37^{o}C$ for few minutes. Preparations were left standing till the following day when they were filtered.

Animal Experiment

Two fully matured female rabbits (*Oryctolagus cuniculus*) obtained from Lagos, Mushin Market were used. Rabbit was selected for the experiment for its longer and larger intestine which will indicate size or weight change easily than laboratory mice. They were fed with carrot for three days, anaesthetized with diethyl ether and dissected. The intestine was removed and placed in a beaker solution of Na_2CO_3 and $NaHCO_3$ at pH 7 for 5 minutes. The intestine was cut into 36 pieces each of 5cm long and reinserted into the beaker solution of Na_2CO_3 and $NaHCO_3$ to maintain pH. Each piece was inserted into varying concentrations (20, 40, 60, and 80%) of chloroform and water extracts of population samples of *I. triloba* at various time intervals (30, 60 and 90 minutes) respectively.

Laboratory Test

Phytochemical analyses were adapted from Swain (1966) to detect the presence and quantities of Tannin, Saponin, Cardiac glycoside and Phenol.

Tannin

50ml of distilled water was added to 10g of sample in a 250ml conical flask, mixed and stoppered with a rubber plug. The preparation was incubated in a water bath for 2-3 hours at $37^{o}C$, allowed to cool and filtered. Dennis reagent and 10ml Na_2CO_3 were added to 10ml filtrate and diluted with distilled water. The preparation was mixed and read at 760nm wavelength after 30 minutes. Tannic acid standard solution was prepared likewise (Swain, 1966).

Saponin

0.5g of of crude extract was dissolved in 10ml of distilled water in a test tube. The tube was stoppered and shaken vigourously for 30minutes. The test tube was allowed to stand vertically for 30minutes. The persistence of a honey comb froth above the surface of the liquid for more than 30minutes indicate the presence of saponin (Swain, 1966).

Cardiac glycoside

0.5g of extract was dissolved in 2ml of chloroform and concentrated H_2SO_4 was carefully added to form a lower layer. The formation of a reddish brown colour at the interface indicates the presence of a steroidal ring, that is, the aglycone portion of the glycoside (Swain, 1966).

Total Phenol

20ml chloroform extract filtrate of sample was put in 100ml conical flask. 2ml of concentrated Acetic acid was added with gentle warming and allowed to cool. 10 drops of diluted H_2SO_4 (1:1) was then added and mixed. 5 drops of 10% $NaNO_2$ was added, mixed and allowed to stand for 5 minutes. The solution was washed with 20ml alcoholic NH_4OH (4:6) solution. The mixture was then cooled in an ice bath and allowed to stand for 1 hour. It was made to volume with alcoholic NH_4OH. The precipitate was filtered off leaving the yellow solution which was read at 420nm with UV visible spectrophotometer. 0-0.01% Phenol standards in chloroform was prepared and treated as above (Swain, 1966).

Infrared Spectroscopy (IRS)

20ml of extract was heated to dryness in a test tube to remove chloroform. The residue was dissolved in hexane and read with IRS. The results were compared with established standard bands and estimates.

Results

The 18 populations of *I. triloba* sampled were realig-

Table 1: Effects of chloroform extract of population samples of Ipomoea triloba on cut pieces of intestine of rabbit

Extract (%)	GpI_1	I_2	I_3	I_4	II_1	II_2	II_3	II_4	III_1	III_2	III_3	III_4	IV_1	IV_2	IV_3	IV_4
Time(mins)/ Intestine (g)	20	40	60	80	20	40	60	80	20	40	60	80	20	40	60	80
30/Initial	0.6	0.4	0.7	0.8	0.7	0.4	0.6	0.4	0.3	0.3	0.2	0.8	0.8	0.9	0.4	0.5
Final	0.5	0.4	0.6	0.9	0.5	0.3	0.5	0.3	0.4	0.3	0.3	0.9	0.7	0.11	0.6	0.7
60/Initial	0.5	0.4	0.6	0.9	0.5	0.3	0.5	0.3	0.4	0.4	0.3	0.9	0.7	0.9	0.6	0.7
Final	0.4	0.4	0.5	0.1	0.4	0.2	0.4	0.2	0.7	0.6	0.5	0.11	0.9	0.12	0.7	0.9
90/Initial	0.4	0.4	0.5	0.1	0.4	0.2	0.4	0.2	0.7	0.6	0.5	0.11	0.9	0.12	0.7	0.9
Final	0.3	0.3	0.5	0.1	0.3	0.2	0.4	0.22	0.7	0.5	0.5	0.11	0.12	0.14	0.1	0.13
Colour	Dark Green				Light Green				Turbid				Light Brown			
Mix	Unmixed				Unmixed				Unmixed				Mixed			

Table 2: Effects of water extract of population samples of I. triloba on cut pieces of intestine of rabbit

Extract (%)	GpI_1	I_2	I_3	I_4	II_1	II_2	II_3	II_4	III_1	III_2	III_3	III_4	IV_1	IV_2	IV_3	IV_4
	20	40	60	80	20	40	60	80	20	40	60	80	20	40	60	80
30/Initial	0.6	0.4	0.7	0.8	0.7	0.4	0.6	0.4	0.3	0.3	0.2	0.8	0.5	0.9	0.4	0.5
Final	0.7	0.5	0.9	0.11	0.9	0.6	0.9	0.7	0.4	0.4	0.4	0.10	0.6	0.10	0.7	0.8
60/Initial	0.7	0.5	0.9	0.11	0.9	0.6	0.9	0.7	0.4	0.4	0.4	0.10	0.6	0.11	0.7	0.8
Final	0.7	0.5	0.10	0.12	0.12	0.7	0.10	0.9	0.5	0.3	0.5	0.11	0.8	0.13	0.9	0.11
90/Initial	0.7	0.5	0.10	0.12	0.12	0.7	0.10	0.9	0.5	0.5	0.5	0.11	0.8	0.13	0.9	0.11
Final	0.5	0.3	0.9	0.12	0.10	0.6	0.10	0.9	0.5	0.5	0.6	0.12	0.9	0.14	0.10	0.13
Colour	Light Brown				Very Light Brown				Very Dark Brown				Dark Brown			
Mix	Mixed				Mixed				Mixed				Mixed			

ned into four categories, on the basis of observable morphological variations:

I: ±glabrous shoot, ovary and fruit, green-brown stem white flower populations

II: ±glabrous shoot, hairy ovary and fruit, green, brown or maroon-red stem, white or pink flower populations.

III: glabrous shoot, hairy ovary and fruit, maroon-red stems and pink flowers

IV: hairy shoot, ovary and fruit, maroon-red stems and pink flowers.

Cut pieces of intestine (5cm long) of freshly sacrificed rabbit contracted in chloroform extract of Groups I and II populations of *I. triloba* but swelled in Groups III and IV population extracts. Group IV population chloroform-extract generated the greatest swelling at 30 and 90 minutes compared with Group III (Table 1). Pieces of intestines in water extract filtrate of *I. triloba* population samples showed little or no contraction but swelled in all concentrations of all plant population groups, Table 2.

Tannin exhibited three significant variations. It was highest in Group I and II populations (0.546-0.556mgml^{-1}), followed by Group III (0.416mgml^{-1}) and lowest in Group IV (0.29mgml^{-1}), Table 3.

The total phenol content was high in all populations examined (6.06-7.51mgml^{-1}) except Group III which exhibited average values (3.55mgml^{-1}). Group IVa populations in addition to the closely related population groups Ia and II lacked saponin unlike groups Ib and III. Cardiac glycoside was present in all populations. IRS detected phenol peaks (2925-2941cm^{-1}) were observed for all population except group IVb. Other populations of same group (IVa) gave as high as 6mg/ml total phenol content (Fig. 1, Table 3). An unidentified peak at 2000cm^{-1} bandwidth was absent in populations Ib, IIa and IVa.

Table 3: Phytochemical analysis of populations of I.triloba

Population Gp.	Tannin mgml^{-1}	Total Phenol mgml^{-1}	Saponin	Cardiac Glycosides	IRS Phenol peak
Ia	0.546	7.52	-	+++	+
Ib	NA	6.95	++	++	+
II	0.556	6.61	-	+++	+
III	0.416	3.55	+	+++	+
Iva	NA	6.06	-	++	+
Ivb	0.29	NA	NA	NA	-

NA: Not available, -: absent, +: present, ++: strongly present, +++: abundant

Discussion

Ipomoea triloba was used by local traditional medicine practitioners in our area for the treatment of heamorrhoids. Piles are painful swellings in the anal cushion which enlarges and becomes blood swollen due to the congestion of connective vascular tissues in the area. The treatment is aimed at reducing the pain, size of haemorrhoids and itching. Groups I and II populations (=*I. triloba* var. *triloba*, partly) was potent in causing contraction of rabbit intestinal muscles. Identification and separation of these populations from the complex variable species may

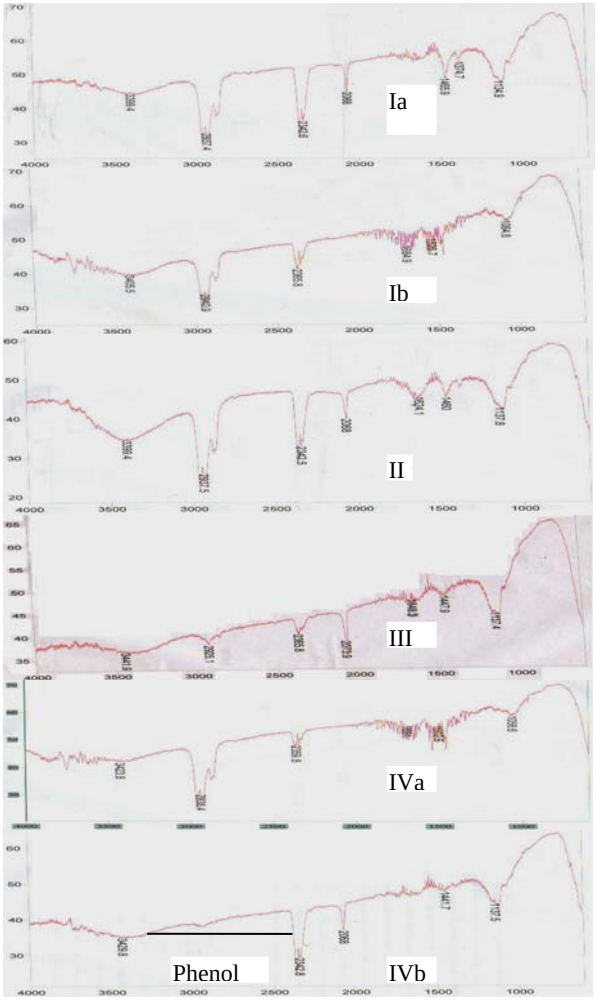

Fig 1: IRS analysis of populations of Ipomoea triloba

produce a more effective treatment than the common practice of using mixed populations.

Phenols may be implicated in the traditional treatment of haemorrhoids by populations of *I. triloba*. It was an active component (6.61–7.52mgml^{-1}) in the biologically active chloroform extract of Groups I & II populations. The impotent Groups III and IV populations had low to average concentrations of total phenol (0-3.55mg/ml). Phenols had been observed to exhibit analgesic activity hence the use of *I. triloba* for headache (Burkil, 1966) and potential pain reliever in the local treatment of haemorrhoids. Whereas, a population in Group IV exhibited high phenolic content (6.06mg/ml), this can only relieve pain without potency for muscle contraction which may be necessary for size reduction of haemorrhoids. Medicinal plants capable of synthesizing phenolic compounds were found to accumulate groups of two to ten chemical elements (Fe, Cr, Cu, Co, Mn, etc.) that are necessary cofactors and activators of many enzymes of phenol metabolism. Some of these medicinal plant species also happen to be overconcentrators of some microelements (Cr, Co, Mn, I) and other chemical elements (Lovkova *et al.*, 1994). Naturally occurring plant phenol, ellagic acid, and its lipophilic derivatives, 3-O-decylellagic acid and 3, 3'-di-O-methylellagic acid, also have antimutagenic action (Smart *et al.*, 1986). Tannins constitute the largest group of water-soluble polyphenolic compounds capable of protein complexation through H-bonding (Kraus *et al.*, 2003). Tannin-protein binding also contributes to the antidiarrhoea and antihaemorrhagic action of the digestive system thereby protecting the digestive organs from injury. Concomitantly, the active population extracts (Groups I and II) contained the highest concentrations of Tannins (0.556 & 0.546mg/ml). Tannin appears to enhance the biological activity of phenols. Removal of tannins from plant extracts had resulted in missing biological activity and specificity of some plant polyphenols (proanthocyanidins and gallic acid/hexahydroxyldiphenic acid esters of glucose) with failure to inhibit ligands binding to specific receptors (Zhu *et al.* 1997).

Taxonomically, the differential pytochemical activity of populations of *I. triloba* confirmed the complex variable nature of the species. This characteristic is useful for classification and identification at intraspecific level. The chemically potent populations (Groups I and II) correspond to *I. triloba* var. *triloba* partly, while the impotent Group IV agrees with *I. triloba* var. *genuina*. Chemosystematic studies in *Sonchus* showed that it could be delimited from related taxa (*Embergeria*, *Babcockia* and *Taeckholmia*) on the basis of absence of luteolin 7-0-rutinoside and apigenin 7-0-rutinoside and weak concentrations of coumarins and luteolin 7-glucoside (Mansour *et al.*, 1983).

Groups Ia and II populations fitted well in the same taxonomic rank, *I. triloba* var. *triloba* both phytochemically and morphologically. Group Ib populations morphologically agree with *I. triloba* var. *triloba* but differs phytochemically with strong saponin presence. Conversely, while group IVa concord with var. *triloba* phytochemically, it differs morphologically belonging to *I. triloba* var. *genuina*. Group IVb could be separated from the entire complex population group on phytochemical parameters but fitted morphologically with var. *genuina*. Hybrids of six *Fuchsia* species have been separated by distinct leaf flavonoid profiles inherited from parents (Williams *et al.*, 1983).

Group III populations as well as some populations Group II reflect features that were inter-

mediate between *I. triloba* vars. *triloba* and *genuina* both phtochemically and morphologically. This population group cannot fit perfectly into vars. *triloba* or *genuina* without error of identification or misplacement. In addition, the high phenolic content of a population in Group IV similar in value to Groups I and II suggests gene flow among the populations of the complex species. Intraspecific hybridisation and introgression might have played a significant role in the variation, diversity and evolution of this complex species.

Acknowledgements

Laboratory assistance by Messrs K. Adetoyi and T. Adedokun of the Basic and Applied Sciences Department, Babcock University are gratefully acknowledged. Part of the work was carried out at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife. The authors are grateful for the technical assistance of Prof. A. Akonnai, the Director and Mr. O. Adisa, the Technologist.

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