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Research Article

Identification of flavonoids in the methanolic and aqueous leaf extracts of *Schrebera swietenioides* Linn.

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Abstract: Plant secondary metabolites provide a scope of developing drugs basing on their specific biological activities. Hence phytochemical and pharmacological screening of medicinal plants has been extensively carried out. In the present paper, flavonoids were isolated from methanolic and aqueous extracts of *Schrebera swietenioides* which is widel used in the treatment of leprosy, diabetes, and liver problems. Chromatographic methods like TLC and HPLC were used for the separation and identification of flavonoids present in methanolic and aqueous leaf extract was studied. The chromatographic methods available for the separation of flavonoids in TLC and HPLC were adopted for the study. Three compounds were identified in TLC study in methanolic and aqueous leaf extracts. In HPLC analysis, peaks corresponding to flavonoids were obtained and were identified by comparing with literature and confirm that methanolic extract contains Rutin, Quercetin and Myricetin where as in aqueous extract Rutin and Quercetin were observed. The isolated compounds were found to be having potent anti diabetic activity. This proves that the anti-diabetic activity of *S. swietenioides* was due to the presence of these flavonoid bio-active compounds.

Keywords: *Schrebera swietenioides*, Flavonoids, The anti-diabetic activity, TLC, HPLC.

Introduction

Schrebera swietenioides belongs to the Oleaceae family and is found in Peru, Tropical and Southern Africa, India and Southeast Asia (Nambia, 1996), (Bosser and Rabevohitra 1985). Commonly known as Weaver's Beam Tree, Banpalas (Hindi), Bullakaya, Magalings, and Tondamukkudi (Telugu). It is a moderate sized deciduous tree, growing up to 20 m tall, with thick grey bark. The roots, bark and leaves are bitter, acrid, appetizing, digestive, thermogenic, stomachic, depurative, constipating urinary astringent and anthelmintic. The fruits are reported to be useful in curing hydrocele. Leaves are pinnate, with 3-4 pairs of opposite leaflets, and a terminal one. Various studies have been conducted on medicinal properties of the plant like antioxidant, anti-Inflammatory and antipyretic activity studied in root (Hansraj, 2009), healing potential in the dexamethasone-suppressed wound healing in rodents (Rajkumar and Bagali, 2010), antidiabetic and antioxidant effect in fruit (Rasal, 2009).

Materials and Methods

Collection of Plant Material:

Fresh leaves of *S. swieteniodes* free from diseases were collected during the month of December from Tirumala hills and different locations of Chittoor District. Taxonomic identification of the plants were carried out with the help of botanists of Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Instrumentation:

Merck silica gel TLC plates (60 F_{254}) were used for TLC separation of plant extracts. HPLC separation was carried on Peak LC 7000 HPLC with LC software, Rheodyne manual injector with 20µl loop, UV detector and Zorbax ODS column (250×4.5mm; 0.5µ).

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Preparation of extracts and preliminary phyto-chemical screening:

The crude extract from leaves of *S. swieteniodes* was obtained using soxhlet extraction method using ethyl acetate, methanol and water solvents. The detailed description of preparation of plant extracts, preliminary chemical screening and quantification methodology was discussed in our earlier publication (Anuradha and Mallikarjuna, 2015).

Biological activity study:

The anti microbial zone inhibition activity of leaves extracts of *S. swieteniodes* were studied by well diffusion method. The anti diabetic activity of crude extracts also studied by fallowing *In-vitro* α- amylase inhibition activity by using visible Spectrophotometer. The detailed description was published in our earlier work (Anuradha and Mallikarjuna, 2016).

TLC separation:

Thin layer Chromatography is a very preliminary analytical method done prior to HPLC and reaction progress can be monitored easily. It can be used for separating compounds from crude extracts and separating impurities from a compound. The Flavonoids present in the methanolic and aqueous extracts of *S. swieteniodes* was separated by different TLC separation methods adopted from available literature. Aluminum foil plates that are coated with absorbent silica powder were used as stationery phase and different solvents were tested. Extracts were separated using mobile phase of benzene: acetic acid: water in the ratio of 125:72:3 (Shweta and Padma, 2012), ethyl acetate - ethanol – water in the ratio of 5:1:5 (Sathish *et al.,* 2008), Butanol:aceticacid:water in the ratio of 125:72:3 (Krishna and Renu, 2013), and Ethyl

acetate: formic acid: water in the ratio of 8:1:1 (Olemy *et al.,* 1994). In all the methods visualization was done in Ultraviolet light observation and separated bands were marked for Rf value calculation.

$$Rf = \text{Distance traveled by compound} / \text{Distance traveled by solvent front}$$

Separation of Flavonoids using HPLC:

To determine the known and unknown flavonoids present in the crude extract, RP-HPLC previously reported methods for Flavonoids were applied. Methanolic and aqueous extracts of *S. swieteniodes* was analyzed using different HPLC methods available in literature. Method 1 was used to separate the flavonoids in gradient elution using mobile phase of methanol: water in the ratio of 1:1 (0-10min), 7:30 (10-20min) at a flow rate of 1.0 ml/min. Separation was achieved on C18 column at run time of 30min (Oliveira *et al.,* 2001). Method 2 consists the mobile phase ratio of (A) methanol and (B) 1% acetic acid/water and the gradient used was 0 min 40%, 10 min 90% B, 15 min 40%, B until 17 min in gradient elution at flow rate of 0.8ml/mi and UV detection at 360nm (Alvarado *et a.l*, 2007). The method 3 consists the separation of flavonoids using Acetonitrile: water (containing 5% TFA) as mobile phase in gradient elution at a flow rate of 1.0 ml/min, UV detection at 254nm on C18 column.

In all the three conditions, methanolic and aqueous extracts of *S. swieteniodes* was injected a volume of 20µL and chromatograms were recorded. The flavonoid compounds were identified by using retention time of compounds adopted from reference methods. After determination of compound, reference library of compounds was performed by using same reference methods with commercially available flavonoids compounds such as flavonoids Rutin, Myricetin, Kaempferol and Quercetin.

Results and Discussions

The solvent extraction and the preliminary screening of phytochemicals (secondary metabolites) is an important step in identification and evaluation of bioactive compounds present in plants. This may lead to medicinal plant drug discovery and development of phytomedicine. Hence solvent extraction with soxhlet extraction apparatus using ethyl acetate, methanol and water solvents were used for extracting phyto-constituents from leaves of *S. swietenioides*. Qualitative analysis for phytochemicals of leaves of *S. swietenioides* indicated the presence of steroids, triterpenoids, carbohydrates, flavonoids, phenols in ethyl acetate extract; steroids, triterpenoids, carbohydrates, alkaloids, flavonoids, phenols in methanol extract and steroids, triterpenoids, saponins, steroidal saponins, alkaloids, carbohydrates, flavonoids and phenols in aqueous extract (Anuradha and Mallikarjuna, 2015). Quantitative estimation confirms the presence of 9.359, 1.446 and 11.404 mg/gram extract of flavonoids in ethyl acetate, methanol and water solvent extracts respectively. Phenolic compounds were found to be 11.42, 10.285 and 16.57mg in ethyl acetate, methanol and water extracts respectively and alkaloids were found to be 16.50 mg/gram and 31.0 mg/gram in methanol and water solvent extracts respectively (Anuradha and Mallikarjuna, 2015). Anti oxidant activity by DPPH inhibition assay, anti microbial activity by agar plate well diffusion method and anti diabetic activity by α- amylase inhibition activity were studied for methanolic and aqueous extracts of *S. swietenioides* and results confirms that the plant is having high free radical inhibition, microbial zone inhibition and anti diabetic activity (Anuradha and Mallikarjuna, 2016).

A novel approach was followed with *S. swietenioides* leaf extracts to determine the biological active compounds by using the chromatographic techniques. In order to determine the number of flavanoids present in the methanolic and aqueous extracts of *S.swietenioides,* liquid chromatographic techniques were applied for separation and identification.Plant extracts were applied to a commercially prepared TLC plate with different solvent systems i.e. the aim of this procedure was to identify the number of components in the extract, distinguish the difference between extract, to find out how close the components of extract are and to develop solvent systems which can further be used for column chromatography. In *S.swietenioides,* ethyl acetate extract has shown the presence of one compound, and three compounds in methanolic extract, and three compounds in aqueous extract respectively. A large number of solvent systems were tried to achieve a good resolution and finally, ethyl acetate/ ethanol/ acetic acid/ water (16:1.5:1:1) and ethanol/ hexane, (90: 10) were confirmed to conduct the tests. The extracts of leaf of each solvent were subjected to TLC. The R_f values are obtained by substituting the values in the above said formula. Ethyl acetate extract of *S. swietenioides* in first mobile phase shown one spot with R_f value 0.62 where as second mobile phase, no spot could be detected. Methanol extract has shown positive response to two mobile phases (one spot in first phase and two spots in second mobile phase) with R_f values of 0.85, 0.93, 0.86 respectively. Three spots were identified in water solvent extract with 0.76, 0.69, and 0.87 as their respective $R_f$values in two selected mobile phases. TLC results were given by figure 1.

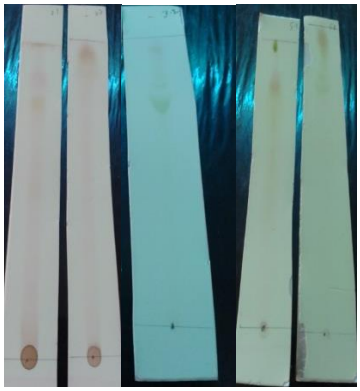

Figure I. TLC results for *S. swietenioides* under UV Light

Typical HPLC chromatograms of the crude leaf extract of shows a good separation of the flavonoid compounds. A blank chromatogram (figure 4.30) with no intense peaks confirms clear mobile phase under selected method condition. All the compounds have been studied for 30minutes of experimental time. Chromatograms showed that different flavonoid compounds separated and eluted at different retention times. Methanolic extract of leaves at condition 1 (figure 2) exhibit no comparative peak at reference retention time. In condition 2 (figure 3), methanolic extract of leaves at exhibited the presence of Rutin (6.34min), Myricetin (7.61min) and Quercetin (8.75min). None of the flavonoid compounds in crude

extracts were found with similar retention time with reference condition 3 retention times, hence no compounds was compared. But the peaks found in the extract may consider as unknown flavonoid compounds. Overall compounds obtained in methanolic extract of *S. swietenioides* leaves are Rutin, Quercetin and Myricetin.

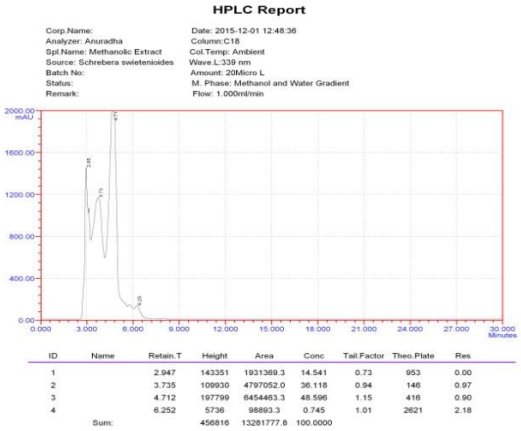

Figure II. Chromatogram obtained for methanolic extract of*S.swietenioides* in condition 1.

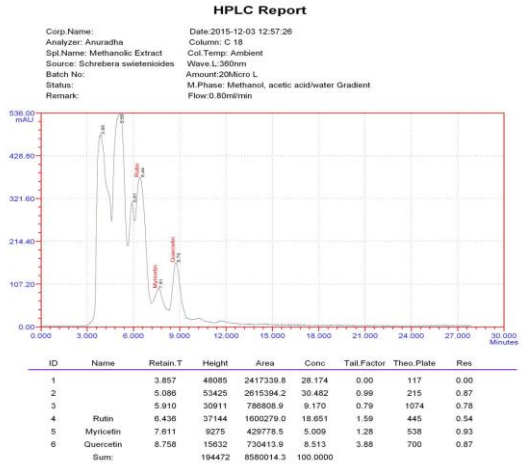

Figure III. Chromatogram obtained for methanolic extract of*S.swietenioides* in condition 2.

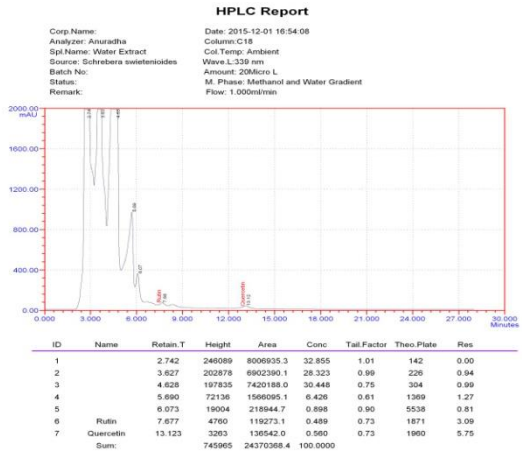

Figure IV. Chromatogram obtained for aqueous extract of *S. swietenioides* in condition 1.

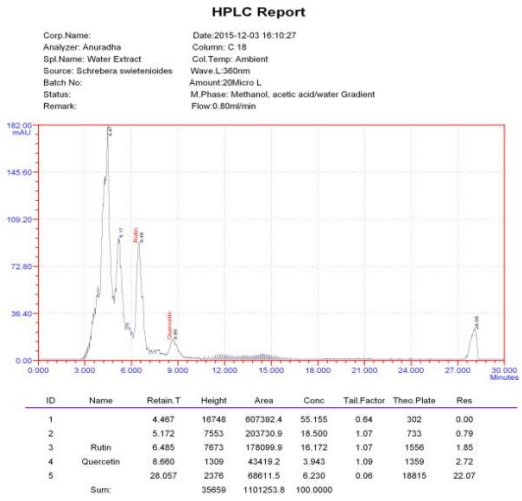

Figure V. Chromatogram obtained for aqueous extract of *S. swietenioides* in condition 2.

In aqueous extract of leaves chromatogram at condition 1 (figure 4) showed the presence of Rutin (7.67min) and Quercetin (13.12min). In Condition 2 (figure 55), the aqueous leaf extract also showed the presence of Rutin (6.48min) and Quercetin (8.66min). With condition 3, the leaf extract showed no flavonoid compounds with similar retention time with reference condition 3 retention times, hence no compounds was compared. But the peaks found in the extract may considered as unknown flavonoid compounds. Overall compounds obtained in aqueous extract of *S. swietenioides* leaves are Rutin and Quercetin. All the identified flavonoid compounds were confirmed by the elution of standard compounds at respective HPLC conditions. Hence, the present investigation proves the presence of active flavonoid compounds and identified as Rutin, Quercetin, and Myricetin.

All separated compounds indicate the presence of various flavonoids in the different extracts of plant. RP-HPLC results indicate the presence of Rutin, Myricetin and Quercetin in Methanolic extract and Rutin and Quercetin in aqueous extract of *S. swietenioides*. The anti-diabetic activity of isolated compounds was confirmed by available literature. Niture *et al.*, (2014) confirmed the Anti-hyperglycemic activity of Rutin. The anti-diabetic activity of Quercetin and Rutin was confirmed by Jadhav and Puchchakayala *et al.*, (2012). Aguirre *et al.*, (2011) and Vessal *et al.*, (2003) confirmed the anti-diabetic activity of Quercetin. The α-glucosidase inhibitory activity of Myricetin was confirmed by Kang *et al.*, (2015). Anti-diabetic effect of Kaempferol was confirmed by Zang *et al.*, (2011). Our *in-vitro* anti-diabetic experimental results are in agreement with published work. Hence all the isolated compounds in *S. swietenioides* were found to be having anti-diabetic activity. This confirms that the anti-diabetic activity of *S. swietenioides* was due to the presence of these bio-active compounds.

Conclusion

The present study confirms the presence of pharmacologically active flavonoid compounds in leaf extracts of *S. swietenioides*. Biological activity results reveal that *S. swietenioides* plants have potential anti-diabetic and antioxidant activity and proves traditional use of these plants for medicinal treatment. The plant leaves can be

applied for pharmacological treatment. Isolation and purification of the bioactive flavonoids may useful for preparation drug compounds for pharmacological treatment of respective diseases.

References

1. Aguirre L, Noemi Arias, M. Teresa Macarulla, Ana Gracia and Maria P. Portillo. "Beneficial effects of quaercetin on obesity and diabetes." The open nutraceuticals journal. 4, (2011):189-198.
2. Anuradha Devi. V and K. Mallikarjuna. "In vitro antimicrobial and antidiabetic activity of leaf Extracts of *Schrebera swietenioides* and *Homalium zeylanicum*". International Journal of life science and Pharma research, 6(3) (2016): 1 – 7.
3. Alvarado A.J., Mauricio Peñarrieta J, Bjorn Akesson and Bjorn Bergenståhl, "Separation of phenolic compounds from foods by Reversed-phase high performance liquid Chromatography". Revista boliviana de química. 24(1) (2007): 1-4.
4. Bosser J, Rabevohitra R. "The genus *Scherebera* found in madagascar, Bull Mus Nation hist nat paris, 4(7), (1985):59-66.
5. De Oliveira Bras H. de, Tomoe Nakashima, José D. de Souza Filho and Fabiano L. Frehse. "HPLC Analysis of Flavonoids in *Eupatorium littorale*" J. Braz. Chem. Soc., 12(2) (2001): 243-246.
6. El-Olemy MM, Al-Muhtadi FJ and Afifi AA. "Experimental Phytochemistry. A Laboratory Manual." (King Saud University Press, KSA) (1994): 8-9.
7. Hansraj Manda. "Antioxidant, anti- Inflammatory and antipyretic activities of ethyl acetate fraction of ethanolic extract of *Schrebera swietenioides*". Roxb. root, IJTPR, 1(1) (2009): 7-11.
8. Jadhav R and G Puchchakayala G. "Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on streptozotocin- nicotinamide induced type 2 diabetic rats". International Journal of Pharmacy and Pharmaceutical Sciences. 4(2) (2012): 251-256.
9. Kang S.J., Jung-Han Yoon ParkHa-Neual and ChoiJung-In Kim. "α-glucosidase inhibitory activities of myricetin in animal models of diabetes mellitus" Food science and biotechnology.24(5) (2015):1897-1900.
10. Nambiar. V. P. K. "Indian Medicinal Plants: A Compendium of 500 Species". 5th edition, Orient Longman Private ltd, Annasalai, Chennai, (1996), pp 88-95.
11. Niture, Ansari and Naik. "Anti-hyperglycemic activity of rutin in streptozotocin induced diabetic rats: an effect mediated through cytokines, antioxidants and lipid biomarkers". Indian J Exp Boil. 52(7) (2014):720-7.
12. Rajkumar S. Bagali, "Evaluation of antidiabetic and antioxidant effect of *Schrebera swietenioides* fruit ethenolic extract". Der Pharmacia Lettre, 2(5) (2010): 278- 288.
13. Rasal AS, "Evaluation of the healing potential of *Schrebera swietenioides* in the dexamethasone-suppressed wound healing in rodents" Int J Low Extrem Wounds., 8(3) (2009):147-52.
14. Sathish K. T., Baskar.R, Shanmugam.S, Rajasekaran.P, Sadasivam.S and Manikandan.V "Optimization of flavonoids extraction from the leaves of *Tabernaemontana heyneana"* Wall., using L16 Orthogonal design, Nature and Science. 6(3) (2008): 10-21.
15. Shweta yadav and padma kumar. "Production, isolation and identification of flavonoids from aerial parts of *Hiptage benghalensis*" international journal of life science and pharma research 2(3) (2012): 1-5.
16. Samariya Krishna and Sarin Renu. "Isolation and identification of flavonoids from *Cyperus rotundus* linn., *in vivo* and *in vitro*" Journal of Drug Delivery and Therapeutics. 3(2) (2013): 109-113.
17. Velavarthipati Anuradha devi and Kokkanti Mallikarjuna. "Screening and quantification of phytochemicals in leaf Extracts of *Schrebera swietenioides* and *Homalium zeylanicum*". International Journal of Pharma and Bio Sciences, 6(4) (2015): 521 – 529.
18. Vessal M, Hemmati M and Vasei M. "Antidiabetic effects of quercetin in streptozocin-induced diabetic rats" Comp Biochem Physiol C Toxicol Pharmacol. 135(3) (2003):357-64.
19. Zang Y, Sato H, Igarashi K. "Anti-diabetic effects of a kaempferol glycoside-rich fraction from unripe soybean (Edamame, Glycine max L. Merrill. 'Jindai') leaves on KK-A(Y) mice". Bio Sci Biotechnol Biochem.75(9) (2011):1677-84.

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