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Thin-layer chromatographic analysis of flavonoids and total phenolics in methanolic and ethanolic extracts of *Senna alata* (L.) Roxb. (Fabales: Fabaceae)

Solomon Oluwole Oladeji

Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria. Email: oladejioluwole@gmail.com.

Abstract. Herbal medicine has proved as a major source of therapeutic agents. They have been used to combat bacterial and fungal related infections and diseases. People depend on medicinal plants such as *Senna alata* (L.) Roxb. (Fabales: Fabaceae) without the knowledge of the chemical composition of the plant. Therefore, there is a need to examine the bioactive components that are present in *S. alata*. The methanolic and ethanolic extracts were obtained using soxhlet apparatus and the concentrated extracts were purified using column chromatography. The fractions were eluted and investigated for the presence of total flavonoids and phenolics. The values of thin-layer chromatographic analysis for the leaf, flower and fruit extracts of *S. alata* when compared to gallic acid and quercetrin (standard) showed that the fractions contained a chemical component in the class of gallic acid and quercetrin, respectively. The presence of total phenolic and flavonoids could be responsible for the observed antifungal and antibacterial activities of the plant. It could be concluded that this plant is a natural source of antimicrobial substances of high importance and are useful in antiseptic and disinfectant formulation as well as in chemotherapy.

Keywords: Herbal medicine; Bioactive compounds; Flavonoids; Total phenolics.

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ORCID
0000-0003-3355-2488
Solomon Oluwole Oladeji

Introduction

Senna alata (L.) Roxb. is a medicinal plant of Fabaceae Family. It is commonly called candle bush, acapulo, ringworm bush and calabra bush. It is found in Ghana and Brazil, but it is now widely distributed in the United States of America and all over Africa, including Nigeria (Kumar, 1984). In many parts of the world, it has long been traditionally used as laxatives and antifungal agents (Farnsworth and Bunyapraphatsara, 1992).

Flavonoids are characterized as containing two or more aromatic rings, each bearing one or more phenolic hydroxyl groups, and connected by a carbon bridge (Beecher et al., 2003). To date, more than 6,000 different flavonoids have been described and the number continues to increase (Harborne et al., 2000). Several classes of phenolics have been categorized on the basis of their basic skeleton: C_6 (simple phenol, benzoquinones), C_6-C_1 (phenolic acid), C_6-C_2 (acetophenone, phenylacetic acid), C_6-C_3 (hydroxycinnamic

acids, coumarins, phenylpropanes, chromones) (Whiting, 2001), C_6-C_2-C_6 (stilbenes, anthraquinones), C_6-C_3-C_6 (flavonoids, isoflavonoids), (C_6-C_3) (lignans, neolignans), (C_6-C_3-C_6)$_2$ (biflavonoids), (C_6-C_3)n (lignins), (C_6-C_3-C_6)n (condensed tannins) (Paiva, 2000).

The growing rate of the activities of microbes increases every day. Virtually everything that surrounds man are contaminated and polluted with these microbes (Beisaga et al., 2011). The advancement in Science and Technology have helped reduced this problems to certain level. In doing this, different researches have been carried out on medicinal plants in order to combat these problems. Despite the recent researches, there is need to ascertain whether it is a specific class of phenolics and flavonoids present in the plants that contributes to the observed bioactivities or the amount of phytochemicals consumed (Indu and Alan, 2010). Therefore, there is a need to determine the bioactive components present in *S. alata*.

The objectives of the research are to extract, isolate and investigate the presence of flavonoids and total phenolics in methanolic and ethanolic extracts of *S. alata* using thin-layer chromatographic analysis.

Materials and methods

Materials and reagents

Glass wares are all clean, well calibrated, analytically graded, in good working condition; rinsed and dried, the chemicals were of analytically grade. Most of the solid reagents used were re-crystallized while the solvents were re-distilled.

Collection of sample

The samples were collected from the Department of Pure and Applied Biology herbarium and was identified and authenticated by the chief technologist of the named department. The samples were collected in batches, rinsed and air dried.

Sample preparation

About 200 g of the samples were accurately sieved using a sieve of mesh size of 20 mm. The sieved samples were stored in air-tight containers for further analysis.

Soxhlet extraction

The extraction was carried out according to the method of Adelowo and Oladeji (2016). 15 g of the pulverized sample was accurately weighed and transferred into the thimble. The refluxing time was 6 h for each extraction. 200 mL ethanol and methanol was used for the extraction. The extracts were obtained in the quick-fit flask and collected using a screw cap collection bottles. This procedure was repeated for other samples.

Concentration

The extracts were concentrated using a rotary evaporator. The six concentrated extracts obtained (ethanolic extracts of leaves, flowers and fruits) also; the (methanolic extracts of leaves, flowers and fruits) were kept in screw cap sample bottles.

Clean up

The column used was made of Pyrex glass, and have small diameter so as to have effective separation and obtain distinctive bands. 0.5 g of glass wool was inserted into the column clamped in a vertical position. 2 g of alumina and sodium sulphate and eluting solvent comprised a mixture of n- hexane and methanol (2:1 vol: 30 mL). The chromatogram was completed when all the eluting solvent has been eluted completely.

Thin-layer chromatography analysis of phenolic compounds

These plates used were pre-coated with silica gel GF254 (Merck 20 x 20 cm). The solvent system used was Petroleum ether: ethanol: Acetone (5:3:1; vol: 18 mL) as the mobile phase. The standard and the samples were spotted on the sample

spotting line, the chromatographic plate with the aid of a micro pipette and it was then air dried for 15 min. After 30 min, the solvent font was reached and the plate was removed and air-dried for 2 min. The plates were then dipped in a chamber that has been saturated with iodine vapour for identification of the spots. After 3-5 min, the plate was removed and the spot detected were circled and the retention factors were calculated.

Results and discussion

The fractions obtained were analyzed on a pre-coated silica gel plate in order to identify the compounds present in each of the fractions obtained from the extracts. The retention factor values were obtained after the development of the chromatogram of sample components. The R_f values for the fractions were then compared with that of standard after detection of the spots in iodine chamber. Table 1 showed the R_f values of quercetrin and gallic acid.

Table 1. The R_f values and mean R_f values of the *S. alata* extracts spotted.

Sample spotted	R_f values (Gallic acid)	Mean R_f values (Gallic acid)	R_f values (Quercetin)	Mean R_f values (Quercetin)
Methanolic Flower	0.42, 0.43, 0.45	0.43	0.68, 0.71, 0.74	0.71
Methanolic Fruit	0.37, 0.39, 0.43	0.40	0.64, 0.67, 0.72	0.68
Methanolic Leaf	0.36, 0.42, 0.44	0.41	0.64, 0.72, 0.75	0.70
Ethanolic Flower	0.39, 0.44, 0.47	0.43	0.66, 0.71, 0.73	0.70
Ethanolic Fruit	0.33, 0.41, 0.44	0.39	0.61, 0.66, 0.75	0.67
Ethanolic Leaf	0.36, 0.39, 0.44	0.40	0.64, 0.69, 0.74	0.69
Gallic acid*	0.41, 0.44, 0.45	0.43	-	-
Quercetin**	0.68, 0.73, 0.76	0.72	-	-

Where the * represent the phenolic standard, and ** represent flavonoid standard.

The thin-layer chromatographic analysis in Table 1 showed that different phenolic compounds, flavonoids and phenolic acids were present in the investigated fractions. The values compared to gallic acid and quercetrin showed that the fractions contained a chemical component in the class of gallic acid and quercetrin respectively. A largest number of flavonoids (quercetin and some unidentified flavonoid-glycosides) and phenolic acids (gallic acid, chlorogenic, caffeic, coumaric and vanillic acid) were found in methanol and ethanol fractions. Rutin and some unidentified flavonoid-glycosides are present in the ethanol and methanol fractions. The ethanol and methanol fractions also contain coumaric, caffeic and chlorogenic acid (Owoyale et al., 2005). The results of the Thin-layer Chromatography analyses showed that different flavonoids and phenolic acids were present in the investigated fractions, this agree with Owoyale et al. (2005) that discovered that there are numbers of flavonoids (rutin, quercetin and some unidentified flavonoid glycosides) and phenolics (chlorogenic, caffeic, coumaric and vanillic acid) in both the ethanolic and methanolic extract of *S. alata*. The compound obtained was suggested to be a flavonoid as the detected spot(s) exhibited light yellow appearance on silica gel plate. This completely agree with Rahaman et al. (2006) research work in which after the determination of the light yellow spots, they sprayed with ceric sulphate reagent to obtain a deep yellow colour which indicate the presence of flavonoid (Figure 1).

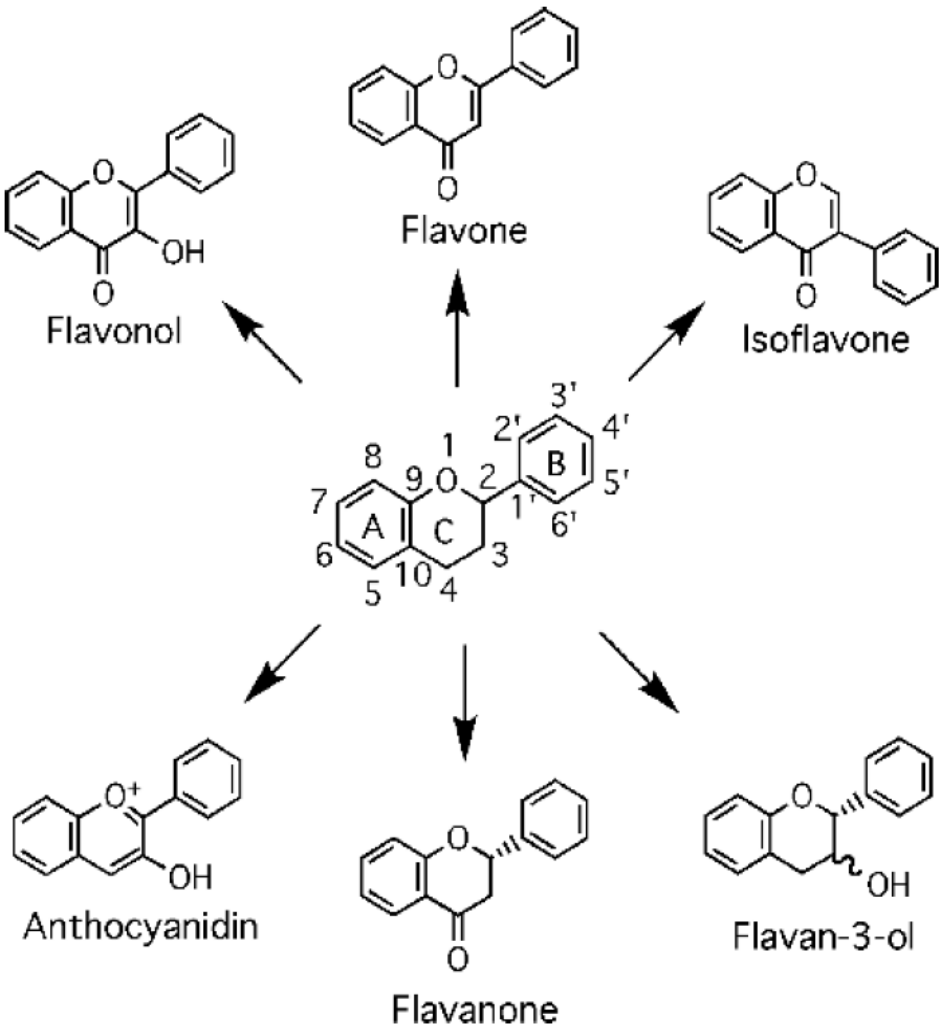

Figure 1. The structures of the classes of flavonoid (Crozier et al., 2000).

Conclusion

The methanolic and ethanolic extracts of *S. alata* showed the presence of flavonoids and total phenolics. The methanolic extracts showed a higher value when compared with their corresponding ethanolic extracts due to the effect of the polarity of the solvent. The results of the study suggested the great value of the plant for use in pharmacy; therefore, it could serve as a natural source of antimicrobial substances of high importance.

Conflict of interest

The author declares that they have no competing interests.

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