The Bioscan 11(3): 1521-1523, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

SPECTROPHOTOMETRIC ANALYSIS OF FLAVONOIDS IN ETHANOLIC LEAF EXTRACT OF *SENNA ALATA* PLANT

OLUWOLE OLADEJI* AND FUNMILAYO ADELOWO
Department of Pure and Applied Chemistry,
Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria
e-mail: oladejioluwole@gmail.com

KEYWORDS
Antifungal
Antibacterial
Bioactive components
Eluted
Spectrophotometric analysis
Prominent bands

Received on :
28.06.2016

Accepted on :
13.08.2016

***Corresponding author**

ABSTRACT

Senna alata is a medicinal plant basically used as antifungal and sometimes as antibacterial. Therefore, there is a need to ascertain this claim by examining the bioactive components that are present in the plant. This research is aimed at determining the chemical components in the leaf of *Senna alata*. The ethanolic extract was obtained using soxhlet apparatus and is screened using thin-layer chromatographic and FT-IR spectroscopic analyses. The ethanolic fraction of the crude extract of leaves of *Senna alata* showed a close retention time when compared with quercetin. The broad band in the spectrum shows the presence of an O-H bond at 3500cm^{-1} due to mesomeric effect of electron withdrawing group. The weak band of 2972.31 cm^{-1} and 2924.09 cm^{-1} indicated the presence of aryl C-H stretching of the flavonols aromatic ring, 1454.33 cm^{-1} indicated the presence of C-O stretching of ketone on carbon one (C_1) and 1649.14 cm^{-1} confirmed the presence of a conjugated carbonyl group (C=O stretch) of ester. The compound obtained suggested to be a flavonoid which could be responsible for the antimicrobial activities of the plant.

INTRODUCTION

Senna alata is a medicinal plant of Leguminosae family. It has many common names such as Candle bush, Acapulo, Ringworm bush and Calabra bush. In the Southwest of Nigeria; *Senna alata* is called Ewe Asunwon Oyinbo'. *Senna alata* plants contain bioactive compounds (phytochemicals) that dictates its therapeutic potency such as flavonoids, alkaloids, tannins amongst others. Flavonoids are characterized as containing two or more aromatic rings, each bearing one or more phenolic hydroxyl groups, and connected by a carbon bridge. They are classified into flavones, isoflavones and isoflavonones, flavanones, flavanols, anthocyanidins, chalcones and dihydrochalcones (Marby *et al.,* 1970). Flavonoids which are widespread in the plant kingdom serve specific functions in antimicrobial activities, flower pigmentation, UV-protection, plant defense against pathogens and legume nodulations (Dixon *et al.,* 2012).

The plant is very important in many areas of life. Different parts of the plant are reported to exhibit several therapeutic properties, such as antibacterial, antifungal, antimicrobial and analgesic (Makinde *et al.,* 2007). The leaves of this plant are used in the treatment of ringworm and skin infections in man (Igoli *et al.,* 2005), hemorrhoids, constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes (Herman *et al.,* 1978; Makinde *et al.,* 2007), as laxatives and antifungal agents (Farnsworth and Bunyapraphatsara, 1992). The FTIR spectroscopic analysis of leaf extract of Senna alata show the presence of flavonoids such as flavonol, flavones and anthocyanidins (Adelowo and Oladeji, 2016).

The acceptance of traditional medicines as an alternative form of health care has led researchers to investigate the antimicrobial activity of medicinal plants (Malwal and Sarin, 2009; Sharma *et al.,* 2013). Herbal medicines are used in areas for health care program in several developing countries such as Nigeria, India, China and some part of Europe. Due to different outcomes on herbal plants, plant products surfaces all over the world due to the belief that they are known to be free from health and environmental effects. Despite these advantages over synthetic drugs, people depend on these plants without the knowledge of the bioactive components (phytochemical compounds). Therefore, there is need to determine the active compound(s) in Senna alata leaf using thin-layer chromatographic and FTIR spectroscopic analyses.

MATERIALS AND METHODS

Collection of sample

The leaf sample was collected between June and July, 2015 from the herbarium of the department of Agronomy, LAUTECH, Ogbomoso. They are identified and authenticated at the herbarium unit of the Department of Pure and Applied Biology, Lautech, Ogbomoso.

Sample preparation and extraction

The leaf sample was air-dried for 25 days, pulverized and sieved using a sieve of mesh size of 20 mm. The sieved samples were stored in air-tight containers and stored at 4ºC for further

analysis. Soxhlet apparatus was used for the extraction and was carried out based on the method of Adelowo and Oladeji (2016) with little modification.

Clean up and qualitative analysis

The column used was made of Pyrex glass, and have small diameter so as to have effective separation and obtain distinctive bands. This was carried out according to the method of Adelowo and Oladeji, (2016).

The 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. This was carried out based on the method of Oladeji et al., (2016).

FT-IR spectroscopic analysis

The fractions were analyzed to determine the functional groups and to elucidate the structure of the compounds using Fourier Transformed Infrared Spectroscopy. FT-IR spectrum of the sample was taken in KBr pellets using Shimadzu FT-IR 8300 Spectrophotometer according to the method of Adelowo and Oladeji (2016).

RESULTS AND DISCUSSION

The results of the retention time analysed after the development of the chromatogram and detection of the spot in iodine chamber are given in Table 1. 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.

From table I, the retention time of the ethanolic fraction and standard calculated from equation (1) below showed a close retention time (R_f) when compared to quercetin (standard used). The compound obtained suggested to be a flavonoid as the detected spot(s) exhibited light yellow appearance on silica gel plate when sprayed with ceric sulphate reagent which indicate the presence of flavonoid. This completely agrees with Rahaman *et al.* (2006) and Oladeji *et al.,* 2016.

FT-IR Analysis of *Senna alata* leaf

The therapeutic potency of *S. alata* is due to the presence of the important functional groups. The functional groups present were determined using FTIR spectrophotometer.

From Fig. 1, the peaks (bands) and their functional group is shown in Table 2. Flavonols are known to possess a characteristic structure containing an aromatic ring cross-linked together. The broad band in the spectrum shows the presence of a O-H bond. The O-H bond indicated the presence of hydrogen bonding. The stronger the hydrogen bond, the longer the O-H bond, the broader and more intense the absorption bands. The O-H group of the flavonol absorbed at 3500cm^{-1} and the reduction in the wave number can be linked to the mesomeric effect of the hydroxyl group with the electron withdrawing group such as C=O. Also, the weak band of 2972.31 cm^{-1} and 2924.09 cm^{-1} indicated the presence of aryl C-H stretching of the flavonols aromatic ring.

The sharp band at 1454.33 cm^{-1} indicated the presence of C-O stretching of ketone on carbon one (C1) and the wave number of 1649.14 cm^{-1} confirmed the presence of a conjugated carbonyl group (C=O stretch) of ester. The C=O of flavonol (Fig. 2) is in conjugation with the O-H bond, thereby shifting the wavelength to 1649.14 cm^{-1}. The bands and their respective functional groups for Flavonol are given in table I.

Flavonoids are the largest group of polyphenolic compounds. They are widely distributed throughout the plant kingdom. 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.,*

Table 1: The R_f values and mean R_f values of the ethanolic leaf and quercetin

Sample spotted	R_f values	Mean R_f values
Quercetin	0.64, 0.69, 0.74	0.69
Ethanolic Leaf	0.36, 0.39, 0.44	0.40

Table 2: The bands and the corresponding functional groups obtained from the spectrum 1 for flavonol analysis

Band (cm^{-1})	Functional group
3500.00	O-H stretch
2972.31	C-H stretch
2924.09	C-H stretch
1923.03	C=C stretch
1649.14	C=O stretch
1454.33	C-O stretch

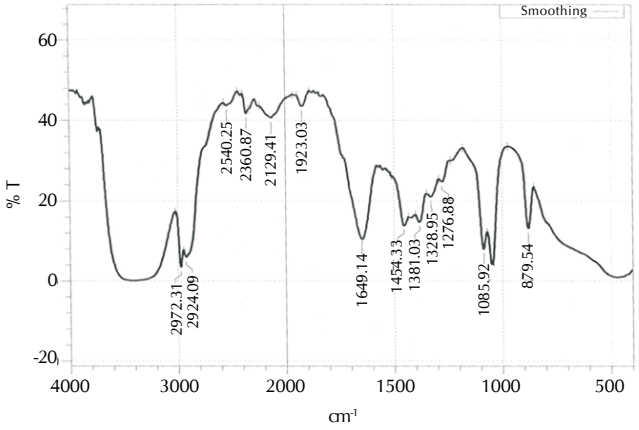

Figure 1: The FTIR spectrum of ethanolic leaf

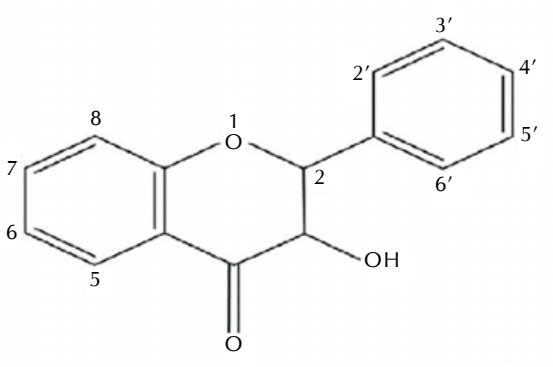

Figure 2: Flavonol structure

2003; Harborne *and* Williams, 2000). They are widespread in the plant kingdom; serve specific functions in antimicrobial activities, flower pigmentation, UV-protection, plant defense against pathogens and legume nodulations (Dixon, 1986).

Flavonols are the most widespread of the flavonoids in plant food. They vary in color from white to yellow and are closely related in structure to the flavones. Examples of flavonols are quercetin, kaempferol, and myricetin (Crozier *et al*. 1997; Hertog *et al*., 1992). Flavonoid compounds have been found to exhibit a greater antifungal and antibacterial activity against some human pathogenic fungi and bacteria (Wuthi, 2003). The therapeutic potentials of *S. alata* leaf could be linked to the presence of flavonols (flavonoids) compound; these compounds were known to be active against pathogenic bacteria such as *Bacillus cereus, Staphylococcus aureus* amongst others (Kumar *et al*., 2012).

REFERENCES

Adelowo, F. E. and Oladeji, S. O. 2016. Spectrophotometric analysis of phenolic compounds in *Senna alata*. *Am. J. Adv. Sci. Res.* **3:** 246-253.

Beecher, G. 2003. Overview of Dietary Flavonoids: Nomenclature, Occurance and Intake1. J. Nutr. p. 133.

Crozier, A., Burns, J., and Aziz, A. A. 2000. Antioxidant flavonols from fruits and vegetables: measurements and bioavailability. *Biol. Res.* **33:** 78-88.

Dixon, R. A. 1986. The phytoalexins response: Elicitation, signaling and control of host gene expression. *Biol. Rev.* **61:** 239-291.

Dixon, R. A. Achnine, L. Kota, P. Liu, C. J. Reddy, M. S. and Wang, L. 2012. Molecular Plant Path. **3:** no. 5, p. 371

Farnsworth, N. B. N. and Bunyapraphatsara 1992. Thai Medicinal Plant: Recommended for Primary Health Care System. *Prachachon Company, Bangkok, Thailan*

Harborne, J. and Williams, C. 2000. Advances in flavonoid research since 1992. *Phytochem.* **55:** 481-504.

Herrmann, K. 1976. Flavonols and flavones in food plants: A review. *J. Food Tech.* **11:** 433-448.

Hertog, M. G. L., Hollman, P. C. H. and Venema, D. P. 1992. Optimization of Quantitative HPLC determination of potentially anticarcinogenic flavonoids in fruit and vegetables. *J. Agr. Food Chem.* **40:** 1591-1598.

Igoli, J., Ogaji, O. and Igoli, N. 2005. Tor-Anyiin T.A; Traditional medicinal practices among the Igede people of Nigeria (part II). *Afr. J. Trad. Compl. Al. Med.* **2:** 134-152.

Kumar, A., Shukla, R., Singh, P., Prasad, C. and Dubey, N. 1984. Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innov. *Food Sci. Emerg.* **4:** 575-580.

Mabry, T. J. Markham, K. R. and Thomas, M. B. 1970. The Systematic Identification of Flavonoids. (New York: Springer Verlag))

Makinde, A., Igoli, J., Amal, L., Shaibu, S. and Garbal, A. 2007. Antimicrobial activity of *Cassia alata*. *Afr. J. Biotech.* **6:** 1509-1510.

Malwal, M. and Sanh, R. 2009. Antibacterial activity of *Murraya koenigil* (l..) speng oil on few clinically important bacterial. *The Bioscan.* **4(4):** 635-654.

Mungule, A., Prakash, Z. and Chaturvedi, A. 2008. Antibacterial and phytochemical screening from different part and calli of *Physalis muniuna*. *The Bioscan.* **3(4):** 393-395.

Rahman, M., Yasmin, T., Shaheen, H. L., Shaheen, T., Tabassam, N., and Zafar, Y. 2006. Harvesting genetic diversity in the cotton genome. National symposium on biotechnology for economic prosperity organized by National Commission on Biotechnology, Green Retreat Hotel, Nathiagali, Pakistan. p. 38.

Sharma, R. A., Pallavi, S. and Ankita, Y. 2013. Antimicrobial Screening of Sequential Extracts of *Datura Stramonium* L. *Int. J. Pharm. Sci*. **5:** 401-4.

Solomon Oluwole Oladeji, Funmilayo Enitan Adelowo and Kehinde Abraham Odelade 2016. Mass spectroscopic and phytochemical screening of phenolic compounds in the leaf extract of Senna alata (L.) Roxb. (Fabales: Fabaceae). Brazilian Journal of Biological Sciences, 2016, v. 3, no. 5, pp. 209-219

Wuthi, S. P. 2003. Antifungal Activities of *Senna alata* extracts using different methods of Extraction. *Process Int. Conf. on MAP, Acta Hort.* p. 597.