Bima Journal of Science and Technology, Vol. 5(2) Dec, 2021 ISSN: 2536-6041

PHYTOCHEMICAL PROFILING OF *SOLANUM NIGRUM* AND *SOLANUM SURATTENSE* FROM LOCAL AREA OF DALA AND GWALE, NIGERIA

[1*]HARUNA, H., [2]BAKO, S.P., [1]MUSTAPHA, Y. AND [1]ALIKO, A.A.

[1]Department of Plant Biology, Bayero University Kano
[2]Department of Biological Sciences, Ahmadu Bello University Zaria

* Corresponding Author: hharuna.bot@buk.edu.ng

ABSTRACT

An investigation of phytochemical analysis of some members of the family solanaceae was carried out at the Department of Biological Sciences, Bayero University Kano. The result of the phytochemical screening of members of the Solanaceae family showed the presence of phytochemicals such as, Tannins, steroids, resins, alkaloids saponins, reducing sugars and flavonoids in both species of *Solanum nigrum* and *Solanum surattense* studied. The results of Thin Layer Chromatography showed similarities at R_f values 0.62, 0.78 and 0.90 while differences were found at R_f values 0.50, 0.66, 0.96 and 0.65.The result of HPLC phytochemical profiling showed similarities as well as differences. Similarities between *Solanum surattense* were at the retention time 31 minutes after elution while differences in retention time peaks were found at 15, 19, 21, 23, 33, 39, 38 and 48 minutes at (254nm). Also at 360nm there was also similarity at 31 and 39 minutes after elution and differences at 18, 19, 33, 37, 40 and 43 minutes. Confirmation of the presence of ellagic Acid at retention time 31 minutes in both *Solanum nigrum* and *Solanum surattense* and differences at 15 minutes showed catechol in *Solanum nigrum* and also Tannic acid at retention time 33 minutes at (254nm). At retention time 39 minutes Acety-Salicylic acid was confirmed in *Solanum surattense*. Difference was also found in *Solanum nigrum* at retention time 41 minutes and was confirmed to be Benzoic acid. This research therefore, proved that plant species belonging to the family Solanaceae contain different types of phytochemicals and thus could be utilize as a tool for modern classification.

Keywords: Solanaceae, Solanum, Phytochemicals, Chromatography

INTRODUCTION

The roots of modern classification spread deeply into the history of Solanaceae classification is typical of many large families of flowering plants (Scrophylariaceae) reviewed by Olmstead and Reeves (1995) in having a core genera assigned to the family in essentially all number of allied genera or groups of genera that are alternatively treated as either belonging to the family, belonging to other related families or segregated into their own families. Members of the family Solanaceae often exhibit unusual combination of characters found in another family or lack characters typical of the family. This necessitated the use of molecular data in the classification of members of Solanaceae and often proved most helpful (Olmstead and Reeves, 1995).

For example, according to Rechinger (1958) a plant sample with white flowers and black berry must be identified as *Solanum nigrum* and added that most commonly found flower colour of S*olanum surattense* is purplish.

However, Nasir (1985) reported that white colour flowers are occasionally present.

The plant *Solanum nigrum* complex has been traditionally used as an analgesic, antispasmodic, antiseptic, antidysentric, antinarcotics', emollient, diuretic tonic, and laxative, anticancer, antiulcer and for disorders of neuro-vegetative system (Manoko *et al.,* 2007). This medicinal value is mainly attributed to the alkaloidal contents of the plant. *Solanum nigrum* is especially known for its toxicity because it contains Solanine a neutoxic glycoalkaloid (Abbas *et al.,* 1998). Alkaloids are said to be excellent taxonomic markers by a number of researchers (Jamil *et al.,* 2007).

The chemical profile as expressed by the occurrence of the major categories of secondary metabolites like alkaloids, triterpenes and anthraquinones is remarkably distinctive (Young *et al.,* 1996). Therefore, so far secondary metabolite profile can contribute to the taxonomic position of some members in a family which remains with morphological controversy (Cardoso *et al.,* 2008), it is hence, pertinent to apply the technique in classifying members of solanaceae family. *Solanum surattense* is one of the solanaceae members and is medicinally important plant with high concentration of Solasodine, a starting material for the manufacture of Cortisone (Heiser, 1969). The research work investigates the phytochemical constituents of *Solanum nigrum* and *Solanum surattense* using phytochemical profiling, thin Layer Chromatography and High Performance Liquid Chromatography (HPLC) to determine the taxonomic relationship existing between the two species.

MATERIALS AND METHODS

Study Site

Samples of *Solanum nigrum* were collected from Goron Dutse, Dala Local Government Area (Lat. 12° 00'04.26"N and Log. 8°29'33.17") growing on the field.While *Solanum surattense* was collected from the field in Lakwaya, Gwarzo Local Government Area (Lat 11° 51'42.29"N and Log 7°55'47.13").

Extraction

Extraction of the samples was carried out according to the protocol described by Oyeleke and Manga (2008).

Ethanol Extraction

Fifty grams (50g) of air – dried powder of the leaves of *Solanum* spp was percolated with one liter of ethanol in separate conical flasks and allowed to stand for one week with intermittent shaking. The solution was then decanted and filtered in order to obtain clear filtrate and labeled. The extracts were repercolated twice for two weeks and labeled E2 and E3 respectively. Each extract of the clear filtrate was concentrated using a Rotary evaporator at a room temperature. The residue was weighed and labeled as Ethanol Fraction of *Solanun nigrum* One (EFSNI). The same procedure was used for the remaining species of *Solanum.*

Hexane Fraction

EFRSNI was macerated with 450ml of n – hexane and the procedure was repeated nine times to obtain the residue. Some fractions of the residue were evaporated to dryness using a Rotary evaporator and the residue obtained was labeled as Hexane fraction of *Solanum nigrum* Two (HFSN1). The insoluble fraction was labeled (HFSN2). The

same procedure was also followed for the other species of *Solanum.*

Chloroform Fraction

Hexane fraction of *Solanum nigrum* leaf two (HFSN2) was macerated with 850ml of chloroform. Using 100ml each time for six times for each sample and then followed by 50ml aliquots for five times, the fractions were combined together and evaporated to dryness using Rotary evaporator. The residue obtained was labeled as chloroform fraction of *Solanum nigrum* leaf one (CFSN1) and the insoluble fraction was labeled chloroform fraction of *Tapinanthus dodoneifolius* two (CFSN2). The same procedure was followed for the remaining samples of *Solanum*.

Butanol Fraction

CFTD2 was macerated with 200ml of n – butanol. Using 50ml four times for each sample, the combined extracts were evaporated to dryness using Rotary evaporator at room temperature. The residue obtained was labeled as butanol fraction of *Solanum nigrum* one (BFSN1). The insoluble fraction was labeled as butanol fraction of *Solanum nigrum* two (BFSN2) . The same procedure was followed for the remaining samples of *Solanum.*

Phytochemical Tests

The extracts were subjected to phytochemical tests to determine the groups of natural products present in the plant material.

Test for Alkaloids

Four milligrams per milliliter (4mg/ml) of the ethanol, hexane, chloroform and n-butanol fractions were treated with three drops of the following reagents and observations noted for each reagent (Meyer's reagent, Dragendoffs reagent and Haga's reagent) were recorded. Production of a turbid suspension or precipitate with any of the reagents indicated a positive test for alkaloids (Oyeleke and Manga, 2008).

Test for Tannins

Four milligrams per milliliter (4mg/ml) of the fractions were stirred with distilled water in a conical flask and the filtrate was mixed with Ferric chloride ($FeCl_3$). Blue – black, green or blue – green precipitate indicates a positive test for tannins (Oyeleke and Manga, 2008).

Test for Steroids and Terpenoids

Four milligrams per milliliter (4mg/ml) of the fractions were dissolved in water and petroleum ether respectively. This was followed by the addition of few drops of acetic anhydride and thereafter the addition of concentrated sulphuric acid. Blue – black, green or mixture of these colours indicates the presence of steroids while red, pink or violet colour indicates the presence of terpenoids (Oyeleke and Manga, 2008).

Test for Flavonoids

To four milligrams per milliliter (4mg/ml) each of the fractions a piece of magnesium ribbon was added followed by the addition of concentrated hydrochloric acid drop wise. Colours ranging from orange to red indicate flavones; red to crimson indicates flavonoles while magenta indicates flavonoids (Oyeleke and Manga, 2008).

Test for Saponins

To four milligrams of boiled distilled water, 0.2g each of the fractions was added. The solution was filtered, allowed to cool and the filtrate was shaken vigorously. Honey comb frothes higher or equal to the aqueous layer was recorded as a positive test for saponins (Oyeleke and Manga, 2008).

Thin Layer Chromatography

This was carried out in order to separate and identify the components of the various fractions. The method of Connell (1990) was used for the thin layer chromatography. The surface of the plate had been ready-coated with a thin layer of silica (ready coated). The plant extract was dissolved in methanol and using very small capillary tubes, the TLC plate was spotted. This was left to dry and was placed in a vertical position inside the tank, covered with a lid and run until after the solvent front was reached. The chromatogram was removed, the solvent front was recorded and air dried. The chromatogram was then sprayed with anisaldehyde and methanol mixed with concentrated sulphuric acid as the detecting reagent.

The chromatogram was then placed in an oven at 120^{o}C for 10 minutes in order for the spots to develop. The different R_f values for the spots were then recorded.

HPLC Profiling

The HPLC analysis was achieved by the use of Waters. HPLC system which was equipped with a Waters HPLC 600 pumping system, Waters 2487 Dual absorbance, detector and Waters integrated software. A Jones chromatographic C18 reverse phase column with a particle size of 5µm, and 250X 4.6mm in column dimension was used for separation process at 35^{o}C (column temperature). The mobile gradient solvent systems used were 1ml formic acid in a liter of Milli-Q water (A); 1ml of formic acid in a liter of methanol (HPLC grade) B. A flow rate of 0.8ml /min with an injection volume of 10ml was used. Also another elution of two solvents was used (acetonitrile) A and (0.1% phosphoric acid in water) B. The method was in accordance with that reported by Samee and Vororat (2007). The gradient programe was started at 8% of A for the first 35 minutes and then increased to 22% for the next 10 minutes before bringing it down to 8%. Detection of wavelengths used for the measurements were 254nm and 360nm.The result obtained was compared with the HPLC fingerprint of the standard phenolic compounds obtained.

RESULTS AND DISCUSSION

Phytochemical Profiling in the Solanaceae

The results of phytochemical profiling showed the presence of tannins in n-butanol and water fractions of *Solanum nigrum*, while in *Solanum surattense* they were found to be present in the ethanol and water extract. Steroids and glycosides were found to be present in all the extract and fractions of both *Solanum nigrum* and *Solanum surattense*. Resins were found to be present in the water extract of *Solanum nigrum*, while they were found to be present in n-hexane and water extract of *Solanum surattense*. Alkaloids were found to be present in ethanol and n-butanol fractions of *Solanum nigrum* and *Solanum surattense*, Saponins were found to be present in all extracts and fractions of *S. surattense* and *S. nigrum* except in its water extract. Reducing sugars were found to be present in ethanol, chloroform and water extract of *Solanum nigrum*, while they were found in to be present in n-butanol and water extract of *S. surratense*.

Flavonoids were found to be present in chloroform and n-butanol fractions of *S. nigrum* and *S. surattense* as shown in Table 1. The phytochemical profiling in members of the Solanaceae family studied showed similarity especially with respect to the presence of alkaloids.

Young *et al.* (1996) Reported that the Phytochemical profile for sub-families, as expressed by occurrence of the major

categories of secondary metabolites (alkaloids, iridoids, triterpenes and anthraquinons) is remarkably distinctive. Therefore, Alkaloids are said to be excellent taxonomic markers by a number of researchers (Dinchev *et al.,*2008; Suau *et al.,*2002).

Table 1: Phytochemical Constituents of *Solanum nigrum* and *Solanum surattense*

S/N	Fractions	Tannins	Steroids	Resins	Alkaloids	Saponins	Reducing sugars	Flavonoids
1	Ef (N)	-	+	-	+	+	+	-
2	nhexf (N)	-	+	-	-	+	-	-
3	Cf (N)	-	+	-	-	+	+	+
4	nBF(N)	+	+	-	+	+	-	+
5	H_2O	+	+	+	-	-	+	-
6	Ef(S)	+	+	-	+	+	+	-
7	Nhex(S)	-	+	+	-	+	-	-
8	Cf (S)	-	+	-	-	+	-	+
9	nBF (S)	-	+	+	+	+	+	+
10	H_2O	+	+	+	-	+	+	-

EF – Ethanol fraction, nhexf – Hexane Fraction, CF – Chloroform, nBF – Butanol Fraction
N- *Solanum nigrum.* S- *Solanum surattense.*
\+ Phytochemical present. _ Phytochemical absent.

Results of Thin Layer Chromatography in the *Solanum* Species

The results of thin layer chromatography showed similarities at R_f values 0.62, 0.78 and 0.90. Differences were found at 0.50, 0.66 and 0.96 as seen in Table 2.

Table 2: R_f Value of Spots on thin layer chromatography of ethanol extracts of *Solanum nigrum* and *Solanum surattense* developed with anisaldehyde and methanol.

Species	No. of Spots	R_f Value
Solanum surattense	6	0.50
		0.62
		0.66
		0.78
		0.90
		0.96
Solanum nigrum	04	0.62
		0.65
		0.78
		0.90

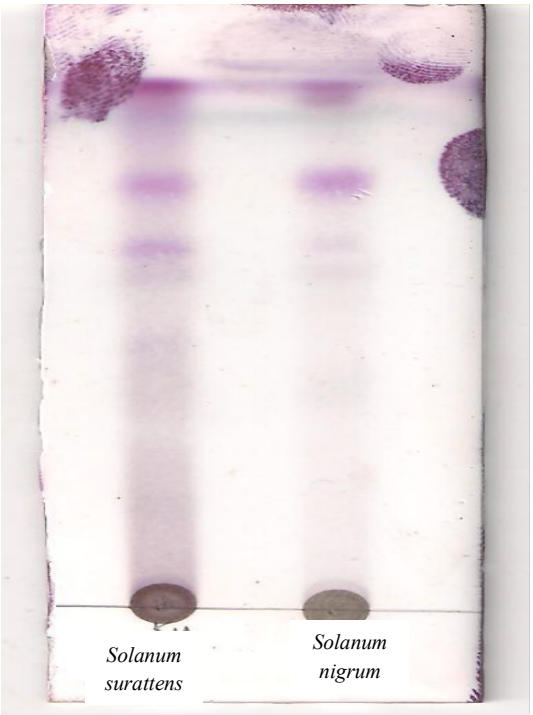

Figure 1: Thin layer chromatography of members of *Solanaceae* showing spots similarities found at R_f values of 0.62, 0.78 and 0.90 while differences were found at R_f values 0.50, 0.66 and 0.96 in *S. surattense* and *S. nigrum*

Phytochemical Profiling of *Solanum nigrum* and *Solanum surattense* Using HPLC

The HPLC Profiling showed the presence of various phenolic substances in *Solanum nigrum* such as catechol at 15 minutes, ellagic acid at 31 minutes, tannic acid at 33 minutes and benzoic acid at 41 minutes in. In *Solanum surattense,* the profiling showed the presence of ellagic acid at 31 minutes and acetyl salicylic acid at 40 minutes (Figure 1 and 2). The presence of various phenolic substances in the present study such as catechols, ellagic acid, tannic acid, benzoic acid and acetyl salicylicacid (Figure 3 and 4) has re-emphasized the importance of *Solanum nigrum* and *Solanum surattense* as medicinally important species of the Solanaceae family.

It has been reported by (Ibrahim *et al.,* 2007; Genash and Venilla, 2011) that these groups of compounds mostly utilized for the purpose of medicinal, therapeutic and taxonomic uses are alkaloids, phenols, glucosemolates, aminoacids, terpenoids, oils and waxes. The fact that *Solanum nigrum* and *Solanum surattense* showed similarity based on the presence of acetylsalicylic acid and ellagic acid showed that there are similarities between these species. It has been reported that acetylsalicylic acid has medicinal values such as analgesic, antipyretic, and antiproliferative, while ellagic acid has antioxidant properties (Festa *et al*., 2001). Therefore, these plants could be considered as also having these properties. In addition, secondary metabolites often play a role in the survival of producing organisms and are known to be involved in plant interactions with animal's, especially herbivores, microbes or even competing higher plants (Wink, 2000).

Thus, similarities in the phenolic substances present in *S. nigrum* and *S. surattense* species could be used to group them into the genus Solanum as they share some common medicinal values. Other researches (Nidhi *et al*., 2012; Abdul-wadood *et al*., 2013 Young *et al*., 1996); which have been previously conducted to support finding of this study and they similarly reported that chemical profile as expressed by the occurrence of the major categories of secondary metabolites like alkaloids, triterpenes and anthraquinones is remarkably distinctive. In addition, Jamil *et* al. (2007) reported that alkaloids are said to be excellent taxonomic markers by a number of researchers.

CONCLUSION

The Solanaceae family studied showed the presence of the various phytochemicals such as tannins, steroids, glycosides, resins, alkaloids, saponins, reducing sugars and flavonoids. The thin layer chromatography of members of the Solanaceae family showed more similarity than differences based on the Rf values of the spots. The HPLC phytochemical profiling of the members of the Solanaceae family also showed similarity and differences at 254nm. Differences were observed with respect with *S. nigrum* which showed the presence of catechol, tannic acid and benzoic acid which confirmed the medicinal potential of the plant. At 360nm the retention time peak showed the presence of acetyl-salycylic acid in both *S. nigrum* and *S. surrattense*, while differences were found with respect to *S. nigrum* which showed the presence of benzoic acid .Considering the fact that there were more differences than similarities, the research work has concluded that each species maintain its status as an independent taxa. The various similarities could be used in the confirmation of species as being a member of the *Solanum* genus. The research work has concluded that each species maintain its status as an independent taxa.

The various similarities could be used in the confirmation of species as being a member of the *Solanum* genus.

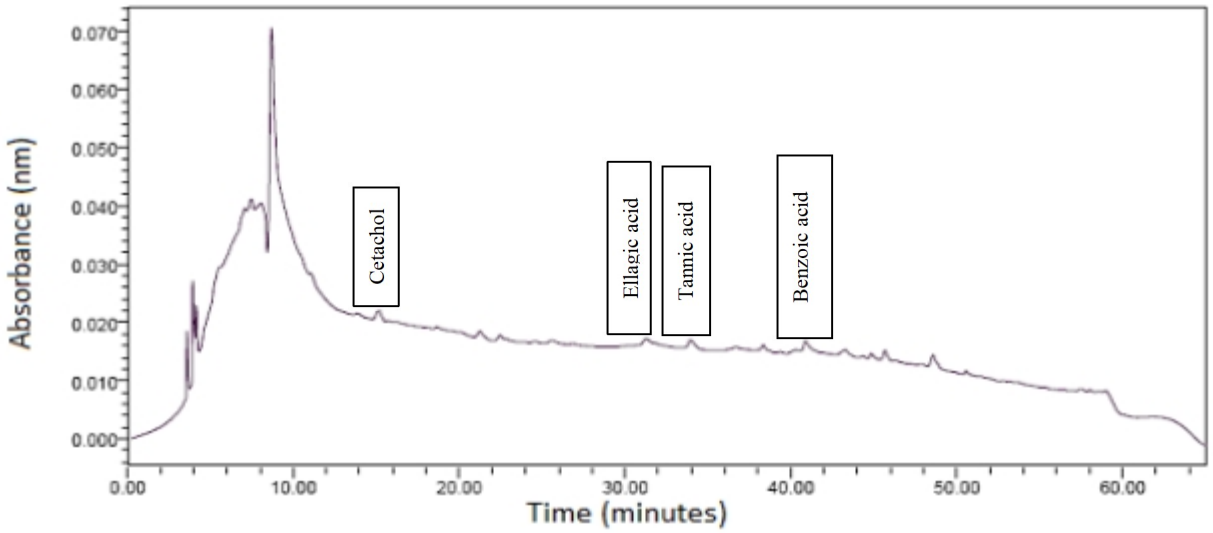

Figure 2: HPLC chromatogram of *Solanum nigrum* at 254nm showing the presence of catechol at 15 minutes retention time, ellagic acid at 31 minutes retention time, tannic acid at 33 minutes retention time and benzoic acid at 41 minutes retention time.

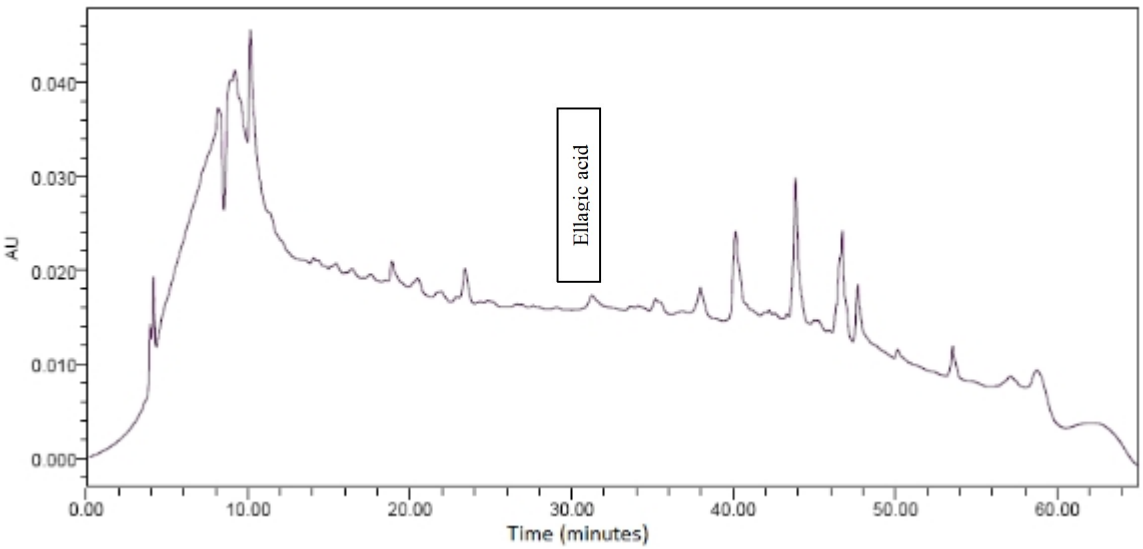

Figure 3: HPLC chromatogram of *Solanum surattense* at 254nm showing the presence of ellagic acid at 31 minutes retention time.

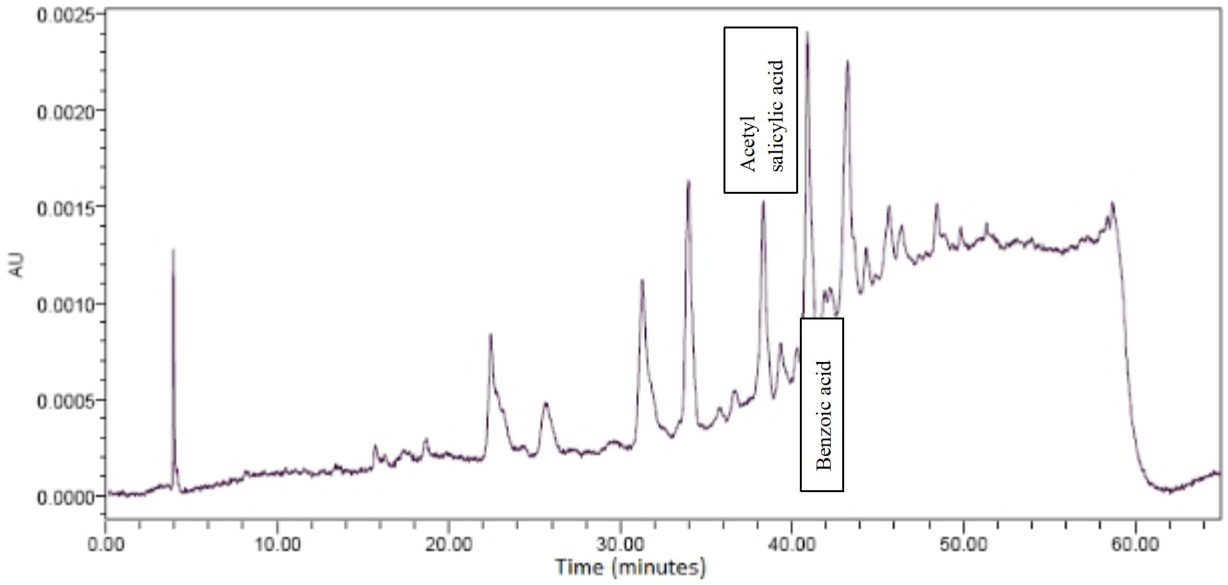

Figure 4: HPLC chromatogram of *Solanum nigrum* at 360nm showing the presence of acetyl salicylic acid at 39 minutes retention time and benzoic acid at 41 minutes retention time.

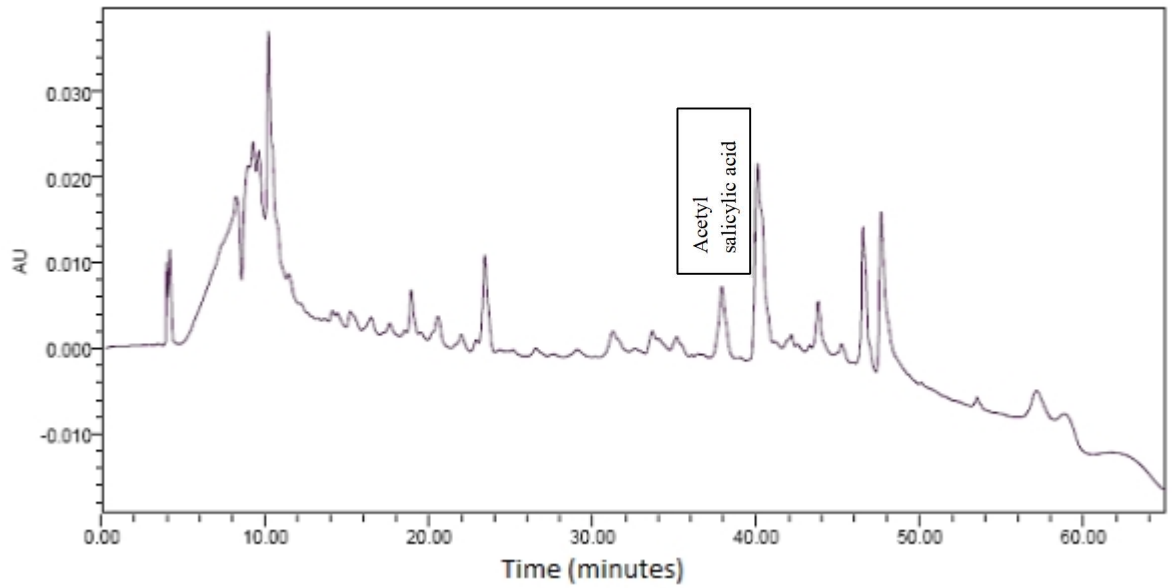

Figure 5: HPLC chromatogram of *Solanum surattense at* 360nm showing the presence of acetyl salicylic acid at 39 minutes retention time.

Recommendation

1. The different phenolic compounds obtained from *S. nigrum* and *S. surrattense* should be evaluated for their medicinal values
2. Further research should look into the application of advance extraction techniques for extraction and quantification of phenolic compounds found in plants especially Solanaceae family

REFERENCES

Abbas, K.H., Paul, R.N., Riley, R.I., Tanaka, T. and Shier, W.I. (1998): Ultra structural effects of AAL-toxin TA from the fungus *Alternaria* on black nightshade (*Solanum nigrum L*) leaf disc and correlation with biochemical measures of toxicity. *Toxic on* 36 (12): 1821 – 1832.

Cardoso, C.L., Silva, D.H.S., Young, M.C.M., Costra, G.I. and de Bolzani, S.V. (2008). Indole chemotaxonomic studies of the Rubiaceae family. *Brazilian Journal of Pharmacy*, 18(1):26-29.

Connell, J.P. (1990). *Natural Product Isolation:* Isolation by Plant Chromatography pp 209-246. Cost of Plant Resistance to Laboratory Chicago; University of Chicago Press Pp. 392.

Dinchtev, D, B., Janda, E.L., Oleszer, W., Aslani, M.R. and Kostova, I. (2008) Distribution of Steroidal Saponins in *Tribulus terrestris* from different geographical regions. *Phytochemistry*, 69(1): 176-186.

Festa, F., Aglitti, T., Duranti, G., Ricordy R., Perticone, P. and Cozzi, R. (2001). Strong antioxidant activity of allegic acid in mammalian cells in vitro revealed by the comet assay. *Anticancer Research*, 21(6A): 3903 – 3908.

Genash, S. and Venilla, J.J. (2011). Phytochemical analysis of *Acanthus illfolius* and *Avicenmia officinalis* by GC-MS. *Res. J. Phytochem*., **5**:60-65.

Heiser, C.B. (1969). *The wonder Berry. In: Night shade paradoxical Plant*. (Ed.) W.H. Freeman-Freman and Co, San Fransisco. Pp 61-105.

Ibrahim, H., Sani, F.S., Danladi, B.H. and Ahmadu, A.A (2007). Phytochemical and anti-sickling Studies of the Leaves of *Hymenocandia acida* Tal. (Eupherbiaceae). *Pak J. Biol. Sci.* 10:788-791.

Jamil, A., Shahid, M., Khan, M.M. and Ashraf, M. (2007). Screening of some medicinal plants for isolation of antifungal proteins and peptides. *Pak. J Bot,* 39(1): 211 – 221.

Linnaeus, C. (1753). Species planetarium. In: Stockholm Murray, London de Quiroz, K, and Gauthier (1992). Phylogenetic taxonomy and the flowering of plant systematic *Bioscience,* 43: 380 – 389.

Manoko, M.K., Van den Berg, R.G. Ferun, R.M.C. Vander Wender, G.M. and Mariari, C. (2007). Aflp markers support separation of *Solanum nodifloum* from *Solanum americanum* sensu strict (Solanaceae) plant. *Syst. Evol.* 267: 1 – 11.

Nasir J.Y. (1985). Solanaceae. In flora of Pakistan (Eds): S.I. Ali and E. Nasir Pakistan Agricultural research council, Islamabad – Fascicle 168: 1-61.

Nidhi, R., Saudhanshu, E.M. and Sandhya, M. (2012). Antioxidant activity of Solanum surattense and Solanum nigrum methanolic extract: an invitro evaluation. Research Journal of Pharmaceutical Dosage Forms and Technology, 4(6): 0975 4377.

Olmstead, R.G and Reeves, P.A (1995). Polyphyletic origin of the Senaphulaniaceae evidence from red and ndhf sequences. *Ann. Missawni Bot. Gard.* 82: 176 – 193.

Oyeleke, S.B and Manga, B.S (2008) Essentials of laboratory practical in microbiology. Tobest publishers.

Rachinger, K.H. (1958). Solanaceae. In flora Iranica (Ed). M Kole and K.H

Rachinger Komission hos Ejnar Munskgaard (publishers). Pp 85 – 88.

Samee, W, Vorarat, S.(2007). Simultaneous Determination of Gallic Acid, Catechin, Rutin, Ellagic Acid and Quercetin in Flower Extracts of *Michelia alba*, *Caesalpinia pulcherima* and *Nelumbo nuafera* by Hplc. *The Pharm Health Science Journal.*

Suau, R., B. Cabezudu, R.Rico, F Najera and J.M Lopez-Romero (2002). Direct Deternunation of Alkanoid Content in *fumaria* Species by GC-MS. *Phytochem Anal*. 13(6): 363-367.

Tetenyi, P., (1987). A chemotaxanomic classification of the Solanaceae. *Ann. Mo. Boz Gard* 74:600-608.

Wink, M. (2000) Interference of alkaloids with neuroreceptors and on channels. In Atta-ur-Rahman (ed). Bioactive natural products, Vol II. Pp 3-129 – Elsevier.

Young M.C.M., M.R. Braga, S.M.C Dierich, V.S. Bolzani, L.M.V. Trovisan and O.R. Gottlieb (1996). Chemosystematic markets of Rubiaceae. *Opera Bot. Belg.* 7: 205 – 212.