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ABSTRACT

For the first time a finger print analysis via high-performance thin layer chromatography (HPTLC) of *Boswellia* Sp. *Burseraceae* was accomplished. A preliminary investigation of the *Boswellia* Sp. *Burseraceae* displayed the presence of chemical constituents that could be involved in the production of innovative pharmaceuticals for an array of antiviral, anticancer, and antibacterial uses. Moreover, the finger print analysis would deem useful for establishing HPTLC standardization for natural and herbal photochemical constituents.

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1. Introduction

For ages, humans have been able to discover and harvest plants for an insurmountable amount of uses (Al-Yasiry and Kiczorowska, 2016, Al-harrasi et al., 2018). Plants have been at the forefront for human discoveries for many ailments related health issues whether they are eaten, inhaled, or for topical uses. They have also led to the development of pharmaceuticals as they contain many chemical constituents relative to the reduction of inflammation and a source of antioxidants. Currently, the extraction of the chemical constituents from plants has allowed researchers to identify specific compounds that would deem useful for the development of new pharmaceuticals and medical treatments.

As there are many plants that possess therapeutic components, one plant in particular via *Boswellia tree* has been in used for centuries (Al-Yasiry and Kiczorowska, 2016). Its common name frankincense has been used for religious practices and its oils as a fragrance (Al-Yasiry and Kiczorowska, 2016; Maloney, 1997; Suhail et al., 2011). Located throughout the Arabian Peninsula, India, and north and east Africa, its resin has been extracted and used for therapeutic practice by herbalist, nutritionist, and doctors (Al-Yasiry and Kiczorowska, 2016). There are mainly four (Thulin,

1987) species of *Boswellia* which includes: *Boswellia carteri*, *B. frereana*, *B. sacra*, and *B. serrate* (Thulin, 1987).

Moreover, *Boswellia* resin are proven to be useful for Alzheimer's disease treatment, suppress cancerous masses, cardiovascular health, used as an analgesic, anti-inflammatory, sued in gastric, hepatic and skin disorders (Coppens, 1995) There are about twenty-five known species in the genus *Boswellia* (Al-harrasi et al., 2018). Some species such as *Boswellia carteri* and *Boswellia serrate* have been used to treat rheumatic disorders, Crohn's disease and ulcerative colitis (Al-Yasiry and Kiczorowska, 2016; Banno et al., 2006). Furthermore, the *Boswellia* species is capable of treating diabetes (Langmead and Rampton, 2006), viruses (Zhang et al., 2013), microbes (Shehata et al., 2011) and cancer (Mothana et al., 2011). More importantly, it has shown to produce anti-neoplastic properties in meningioma cell, leukemia cells, hepatoma cells, melanoma cells, fibrosarcoma cells, colon cancer cells, and prostate cancer cells (Shao, 1998, Liu et al., 2002a, 2002b, Park et al., 2002, Zhao et al., 2003, Ahmed et al., 2014).

In the present study a thorough phytochemical screening of *Boswellia* Sp. *Burseraceae* was done, to test all possible types of chemical species that exist within its aqueous extract. Furthermore, high-performance thin layer chromatography was done to give an extensive finger print analysis of its biochemical constituents.

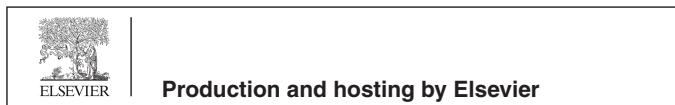
2. Experimental methods

Investigations lead to an array of chemical tests. The experimental analyses included test for the following substances (constituents): alkaloids; amino acids; carbohydrates; flavonoids; glycosides; phenolic compounds; proteins; and then steroids and

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triterpenoids. High performance thin layer chromatography was also done to determine various chemical species present in the *Boswellia* extract.

2.1. Extraction methods

Petroleum ether of 40° to 60°, benzene, chloroform, methanol solutions were obtained by a successive extraction method. Aqueous extract was performed by a maceration method. Furthermore, all extracts were given a proximate chemical analysis (Pang et al., 2009).

2.2. *Boswellia* Sp. *Burseraceae* alkaloid test

To test presence of alkaloids, several tests were done using dilutions of hydrochloric acid with special reagents. These reagents included the Dragendorff's test in which the indication of a reddish-brown precipitate from the extract-HCl solution will be positive for alkaloids. The Mayer's test, will produce a cream-like precipitate to indicate the presence of an alkaloids. Another analysis done, which produces a red- brownish color with the on-set of an alkaloidal presence, is the Wagner's Test. The Hager's test was used to produce a yellow precipitate and the Tannic acid test was also done.

2.3. *Boswellia* Sp. *Burseraceae* amino acid identification

The presence of amino acids was tested by Millon's test in which 2 mL of Millon's reagent was added to the *Boswellia* Sp. *Burseraceae* to produce a white precipitate. The Ninhydrine Test uses the Ninhydrine solution with addition of the extract with the producing a violet color which indicates the presence of the amino acids.

2.4. *Boswellia* Sp. *Burseraceae* carbohydrate testing

The Molisch's test used several drops of an alcoholic α -naphthol solution with the addition of concentrated sulphuric acid added dropwise to the *Boswellia* Sp. *Burseraceae* extract. The purple hue indicated the presence of carbohydrates. A Barfoed's test was done with 1 mL of *Boswellia* extract and Barfoed's reagent heated together. Red cupric oxide formation indicates the presence of monosaccharides and disaccharides. Seliwinoff's test was done by adding resorcinol crystal to *Boswellia* extract. With equal volumes, concentrated HCl was added and heated with a rose color indicates the presence of ketohexoses. *Boswellia* extract was added to hydrochloric acid and heated with the addition of phloroglucinol.

2.5. Identification of flavonoids

Concentrated HCl, was added dropwise to a solution of *Boswellia* extract to perform a Shinoda test. Crimson red or green color indicates the presence of flavonoids. For the alkaline reagent test, sodium hydroxide (NaOH) solution was added dropwise to *Boswellia* extract. After the yellow color appears, diluted acid was added dropwise to give clear appearance. Zinc hydrochloride powder was added to a mixture of *Boswellia* extract. Concentrated HCl was added dropwise to the solution to exhibit a red color.

2.6. Identification of glycosides

200 mg of *Boswellia* extract was diluted in sulphuric acid in warm water bath. After filtration, neutralization of the extract was done with a 5% solution of NaOH. Fehling's solution A and B, 0.1 mL, in a heated water bath for two minutes, was added until

solution reached alkalinity. Red precipitate mass was compared for Test A and Test B.

2.7. Test for anthraquinone glycosides

For the Bontrager's test, *Boswellia* extract and sulphuric acid (1 mL) were heated till boil in test tube for 5 mins. After filtration, the dichlormethane was added and shaken in a dilute solution of ammonia producing a red-pink color on the ammoniacal layer.

2.8. Test for hydroxyl anthraquinones

Boswellia extract sample solution was treated (dropwise) with potassium hydroxide.

2.9. Test for Cardiac glycosides

For the Legal's test, *Boswellia* extract was treated with pyridine and alkaline sodium nitroprusside solution. In Baljet's test, the *Boswellia* extract was treated with picric acid.

2.10. Identification of saponin glycosides

To complete the froth formation test, in a test tube, *Boswellia* extract was placed in 2 mL of water then shaken until foam formation.

2.11. Identification of phenolic compounds

Ferric chloride test was accomplished by treating the *Boswellia* extract with ferric chloride and with vanillin and HCl solutions to reveal the presence of tannin. For the Phenazone test, *Boswellia* extract was added, 5 mL, to 0.5 g of sodium acid phosphate then warmed, 2% phenazone solution was used to filtrate the solution. Gelatin test was completed with 1% gelatin solution with 10% sodium chloride (NaCl) added to *Boswellia* solution which should form a precipitate. For gallotannins testing, *Boswellia* extract was added to potassium iodide (KI) solution. For testing for ellagitannins, *Boswellia* extract was added to solution of concentrated acetic acid and nitric acid solution.

2.12. Identification of proteins

For the Trichloroacetic acid test, trichloroacetic acid was added to *Boswellia* extract sample to produce a precipitate. 1 mL of concentrated nitric acid was added to 5 mL of *Boswellia* extract for the xanthoproteic test. After yellow precipitate is formed, a 40% NaOH solution was added to create an orange hue.

2.13. Identification of steroids and triterpenoids

For the Liebermann-Burchard test, acetic anhydride was added to *Boswellia* extract brought to a light boil then cooled to room temperature. Concentrated sulphuric acid was added to the solution. Concentrated sulphuric acid was added to *Boswellia* extract solution for the Salkowski test.

2.14. HPLC of *Boswellia* Sp. *Burseraceae*

2.14.1. Sample preparation

Boswellia extract was diluted with dimethylsophoxide (DMSO) at a 1:1 ratio followed by filtration. A 10 μ L volume of sample was applied on the thin layer chromatography (TLC) plate of 60 F₂₅₄ (10 cm \times 10 cm) having a 250 μ m thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator

Table 1
Results for identification of phytochemical constituents by HPTLC Analysis of Aqueous Extract of *Boswellia* Sp. *Burseraceae*.

S. No.	Name of the Test	Result
Test for Proteins		
1.	Trichloroacetic Acid Test	NEGATIVE
2.	Xanthoprotein Test	NEGATIVE
Test for Alkaloids		
1.	Dragendroff's Test	POSITIVE
2.	Tannic acid Test	POSITIVE
Test for Amino Acids		
1.	Millons Test	NEGATIVE
2.	Ninhydrin Test	NEGATIVE
Test for Carbohydrates		
1.	Molisch's Test	POSITIVE
2.	Barfoed's Test	POSITIVE
3.	Seliwinoﬀ's test	POSITIVE
4.	Test for Pentoses	NEGATIVE
Test for Flavonoids		
1.	Shinoda Test	NEGATIVE
2.	Alkaline reagent Test	NEGATIVE
3.	Zinc hydrochloride test	NEGATIVE
Test for Phenolic Compounds		
1.	Ferric chloride test	NEGATIVE
Specific chemical test for Tannins		
1.	Test for Gallotannins	NEGATIVE
2.	Test for Ellagitannins	NEGATIVE
Test for Steroid s & Triterpenoids		
1.	Liebermann- Burchard Test	NEGATIVE
2.	Salkowaski test	NEGATIVE
3.	Sulfur powder test	NEGATIVE
General Test for Glycosides		
1.	Test A & B	NEGATIVE
Specific Chemical test for Glycosides		
Cardiac Glycosides		
1.	Legal's test	NEGATIVE
2.	Baljet's test	NEGATIVE
Saponin Glycosides		
1.	Froth formation Test	NEGATIVE
Anthraquinone Glycosides		
1.	Borntrager's test	NEGATIVE
1.	Test For Saponins	POSITIVE
1.	Test For Anthocyanins	NEGATIVE

(Switzerland). Slit dimensions ranged from 6 mm × 0.45 mm. The scanning speed was set at 20 mm per sec. Linear ascending was done in a 10 cm × 10 cm twin through glass chamber (CAMAG, Muttenz, Switzerland). Ethyl acetate: methanol, a 8:2 v/v ratio, was set as the mobile phase. Chamber saturation duration for mobile phase was set for 15 min. Chromatogram length was 8 cm with 20 min development time. TLC plates were air dried with an air blower. CAMAG TLC scanner at 260 nm was used for densitometric scanning (WINCATS software version 1.4.3.) (Kokate, 1996, Chatwal and Anand, 2004)

2.14.2. Instrumental conditions

Spotting was done on bands with 8 mm width having 12 mm space between each band, with a 100 µL sample syringe (Hamilton, Bondaduz, Switzerland) on a precoated silica gel aluminum plate

Table 2
HPTLC profile of *Boswellia* Sp. *Burseraceae* aqueous extract in DMSO.

Sr. No.	Track	Start Position	Start Height	Max Position	Max Height	End Position	End Height	Area	Area %
1	2	0.07 Rf	3.6 AU	0.07 Rf	133.8 AU	0.09 Rf	0.0 AU	904.8 AU	14.83
2	2	0.11 Rf	0.3 AU	0.13 Rf	12.2 AU	0.14 Rf	6.5 AU	165.3 AU	02.71
3	2	0.14 Rf	6.7 AU	0.16 Rf	24.8 AU	0.18 Rf	0.6 AU	519.7 AU	08.52
4	2	0.51 Rf	0.3 AU	0.54 Rf	17.6 AU	0.56 Rf	0.2 AU	383.2 AU	6.28
5	2	0.58 Rf	1.1 AU	0.61 Rf	50.3 AU	0.65 Rf	0.6 AU	1329.3 AU	21.79
6	2	0.68 Rf	0.3 AU	0.73 Rf	15.4 AU	0.76 Rf	2.5 AU	665.5 AU	10.91
7	2	0.79 Rf	0.2 AU	0.83 Rf	64.8 AU	0.87 Rf	2.8 AU	2133.1 AU	34.96

3. Results and discussion

3.1. Test for proteins

To detect presence of the protein materials in the *Boswellia* extract, a test using trichloroacetic acid and xanthoprotein test were done. There was non-indicative precipitate formation resulting from the trichloroacetic acid test. As for the xanthoproteic test, no orange color was formed.

3.2. Test for alkaloids

The Dragendroff's test was performed to test for alkaloids present in the *Boswellia* extract. From result, the brown-like precipitate was formed from the reagent. This indicated that the *Boswellia* extract has alkaloids present. Furthermore, the tannic acid test further confirmed the presence of alkaloids as the precipitate formed in from the solution.

3.3. Test for amino acids

The Million's test did not result in the formation of a white precipitate giving an unclear result for amino acids. Results for the ninhydrine test were negative for a violet indicator hue, further indicating that amino acids were not present in the *Boswellia* extract.

3.4. Test for carbohydrates

The violet hue for the Molisch's test was detected indicating that the *Boswellia* extract contains carbohydrates. Also, monosaccharides are present in the *Boswellia* extract as indicated by the Barfoed's test. Moreover, the presence of ketohexoses tested positive per the Seliwinoﬀ's test producing a rose-like hue. The test for pentoses was unclear as no hue was detected.

3.5. Test for flavonoids

The Shinoda test resulted in an unclear reading indicating that no flavonoids are present in the *Boswellia* extract. Moreover, neither the alkaline reagent test nor zinc hydrochloride test exhibited positive reading for the presence of flavonoids.

3.6. Test for phenolic compounds

The Ferric chloride test was used to detect the present of phenolic compounds. The result was negative for the *Boswellia* extract. Furthermore, both the gallotannin and ellagitannin test resulted in negative readings for the presence of tannins in the *Boswellia* extract.

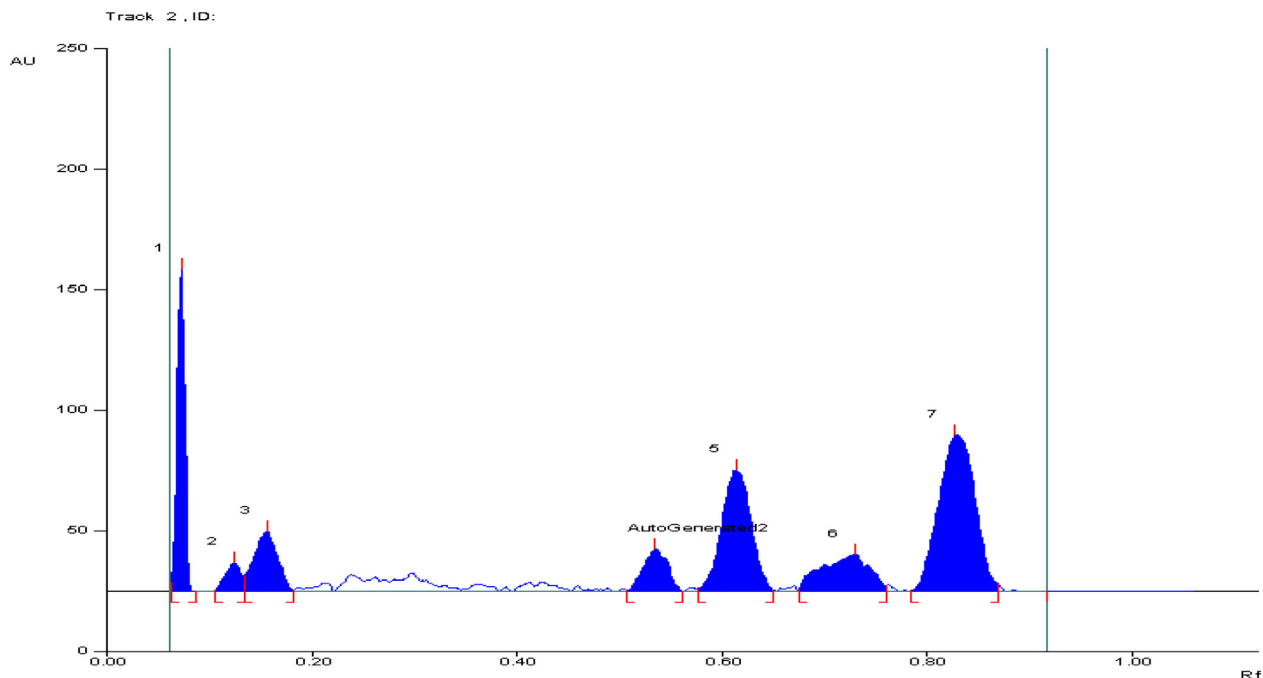


Fig. 1. 2 D chromatogram of aqueous *Boswellia Sp. Burseraceae* extract.

3.7. Tests for Steroid and triterpenoids

The *Boswellia* extract showed negative results for the presence of steroids and triterpenoids. The performed tests were the Liebermann-Burchard test, Salkowski test, sulfur powder test. Each test showed negative results.

3.8. Tests for glycosides

For the general test for the glycosides, no comparison was needed for Test A and Test B. Both resulted in the negative

presence of fundamental glycosides. Cardiac glycosides tested negative for the *Boswellia* extract as so did the Baljet's test. Neither indicates the presence of cardiac glycosides. To test for saponin glycosides the froth formation test was done. It resulted in negative results for the presence of glycosides. The Borntrager's test resulted in negative reading for anthraquinone glycoside as no pink-red hue was indicated. However, positive reading was detected for saponins and negative for anthocyanins (see Tables 1 and 2).

High performance thin layer chromatography (HPTLC) of *Boswellia* extract was done to determine the presence different chem-

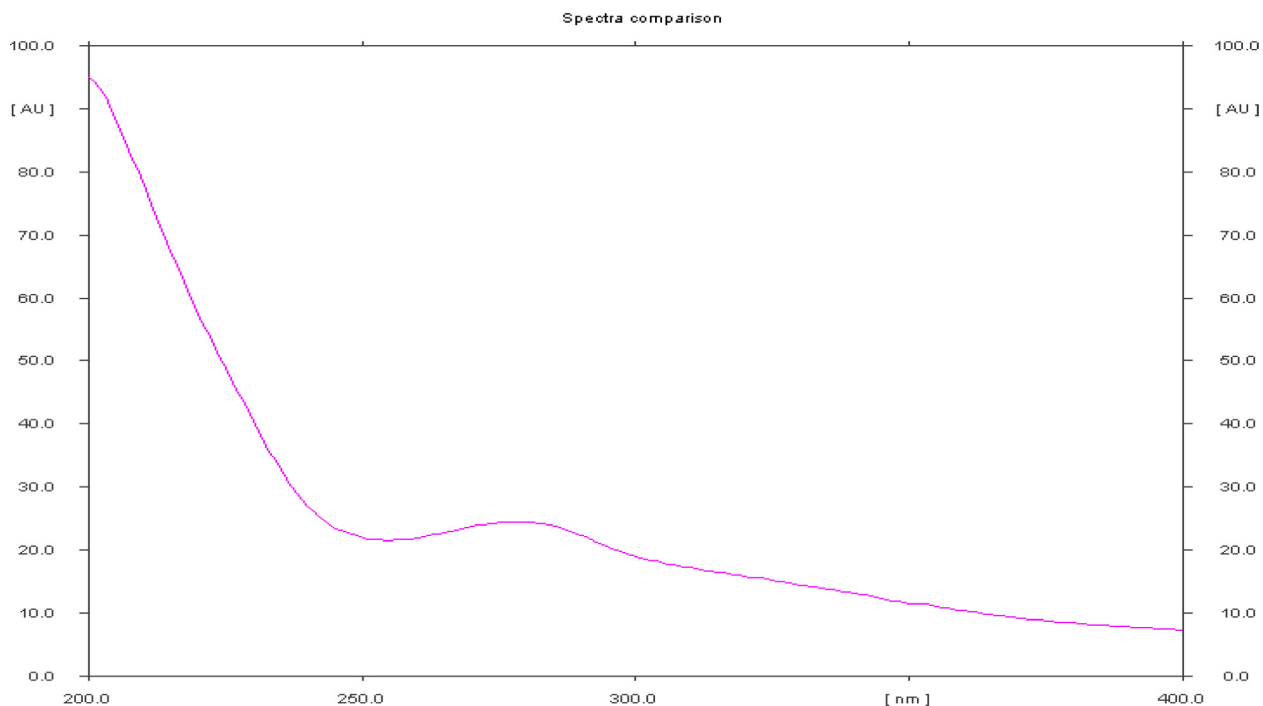


Fig. 2. Spectra of Peak observed at Rf Value 0.07.

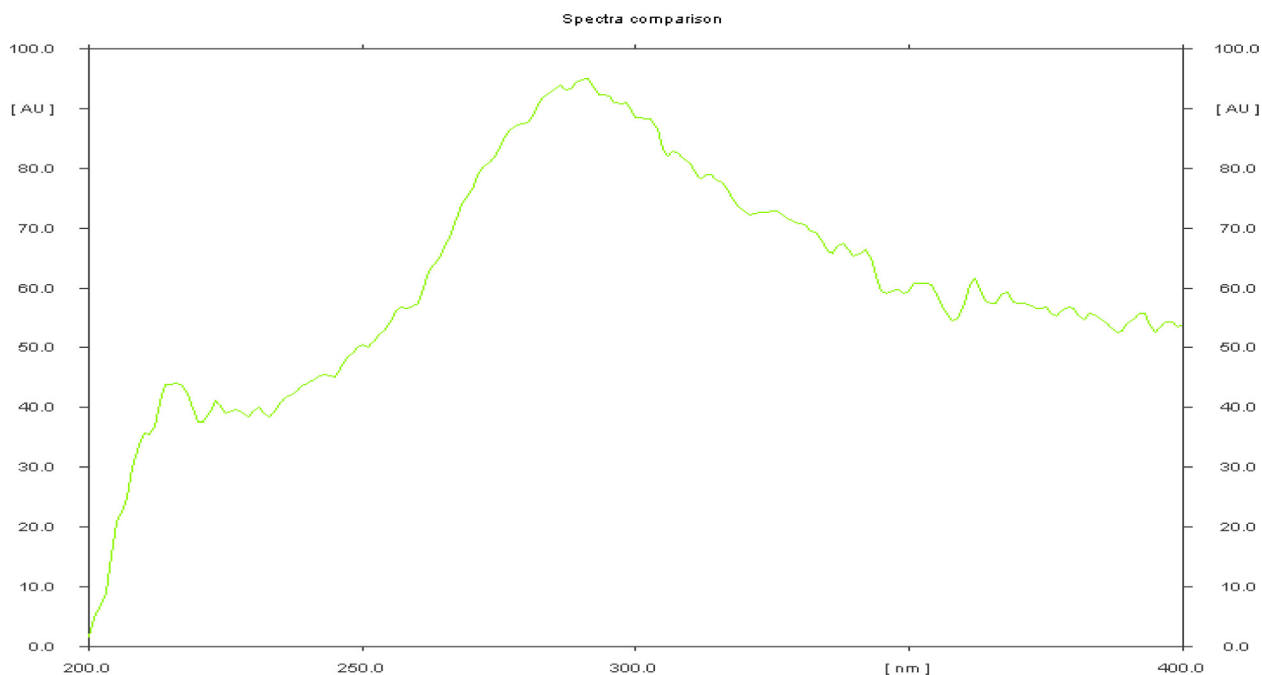


Fig. 3. Spectra of Peak observed at Rf Value 0.13.

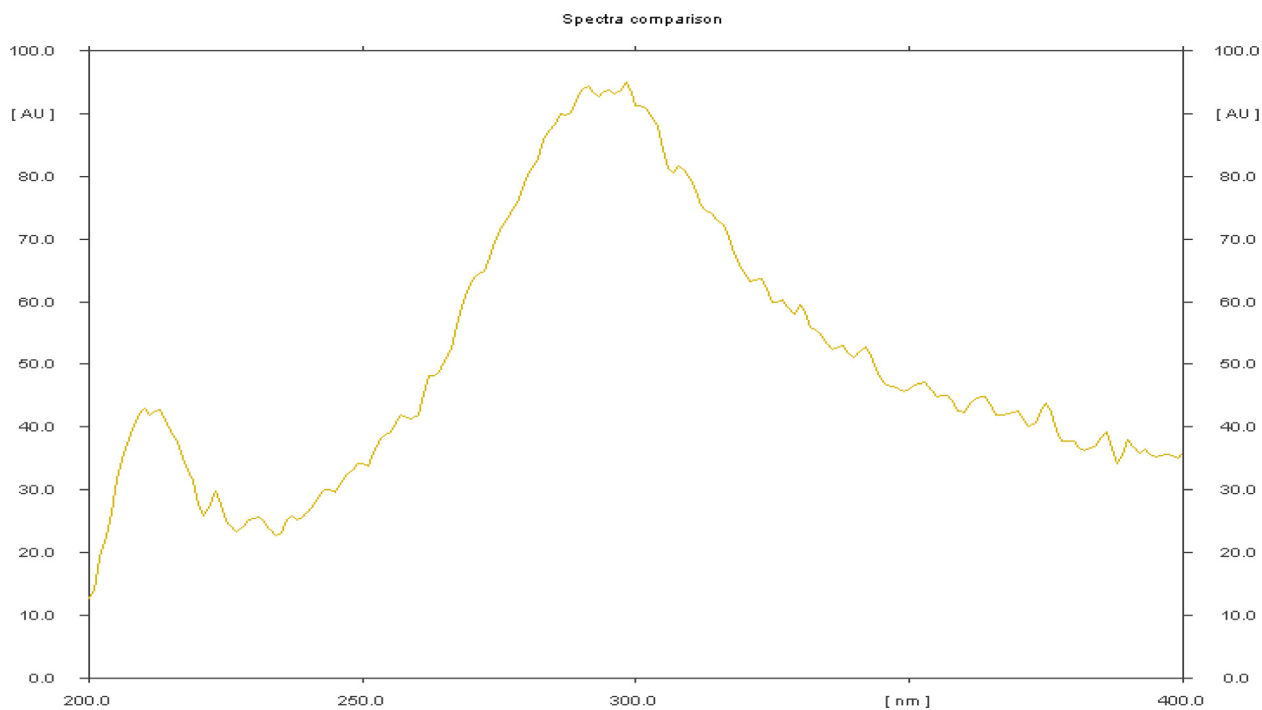


Fig. 4. Spectra of Peak observed at Rf Value 0.16.

ical constituents. HPTLC result in 7 peaks minus the background noise following ultra-violet scan to further confirm HPTLC readings (Fig. 1). The retardation factors ranged from 0.007 R_f to 0.83 R_f . Moreover, area values ranged from 165.3 AU to 2133.1 AU. The largest area percentage was established by R_f value (0.83 R_f) and 34.96% of area. However, the R_f value (0.07) had the second largest area at value of 14.83% and area of 904.8 AU (Table 2). HPTLC permits the examination of phytochemical constituents. The HPTLC finger printing of the extracts further exhibited evidence of phyto-

constituents via Fig. 2. Aside from providing R_f values, HPTLC also establishes data for absorption spectra maximum values which can be observed in Figs. 3–8.

Boswellia Sp. *Burseraceae* extract contains important phytochemical-constituents that deem useful for pharmaceutical and medicinal innovations. Preliminary phytochemical test results indicate the presence of several phytochemical constituents via alkaloids, carbohydrates, and saponins. While some phytochemical constituents are present for this sample, it may be beneficial to

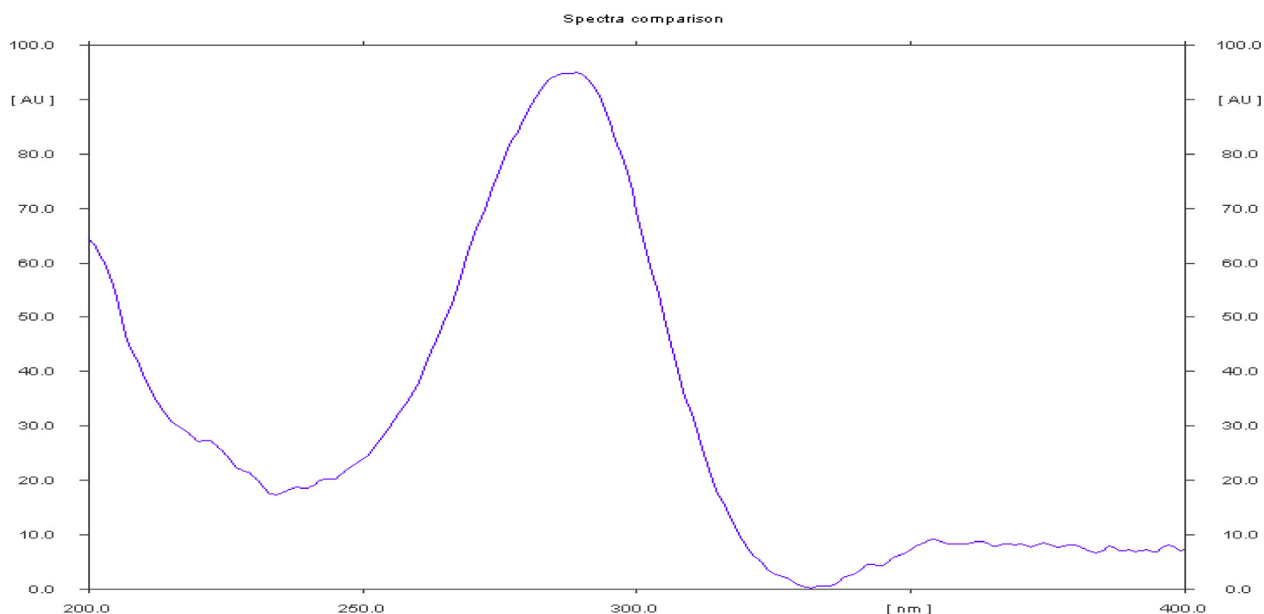


Fig. 5. Spectra of Peak observed at Rf Value 0.54.

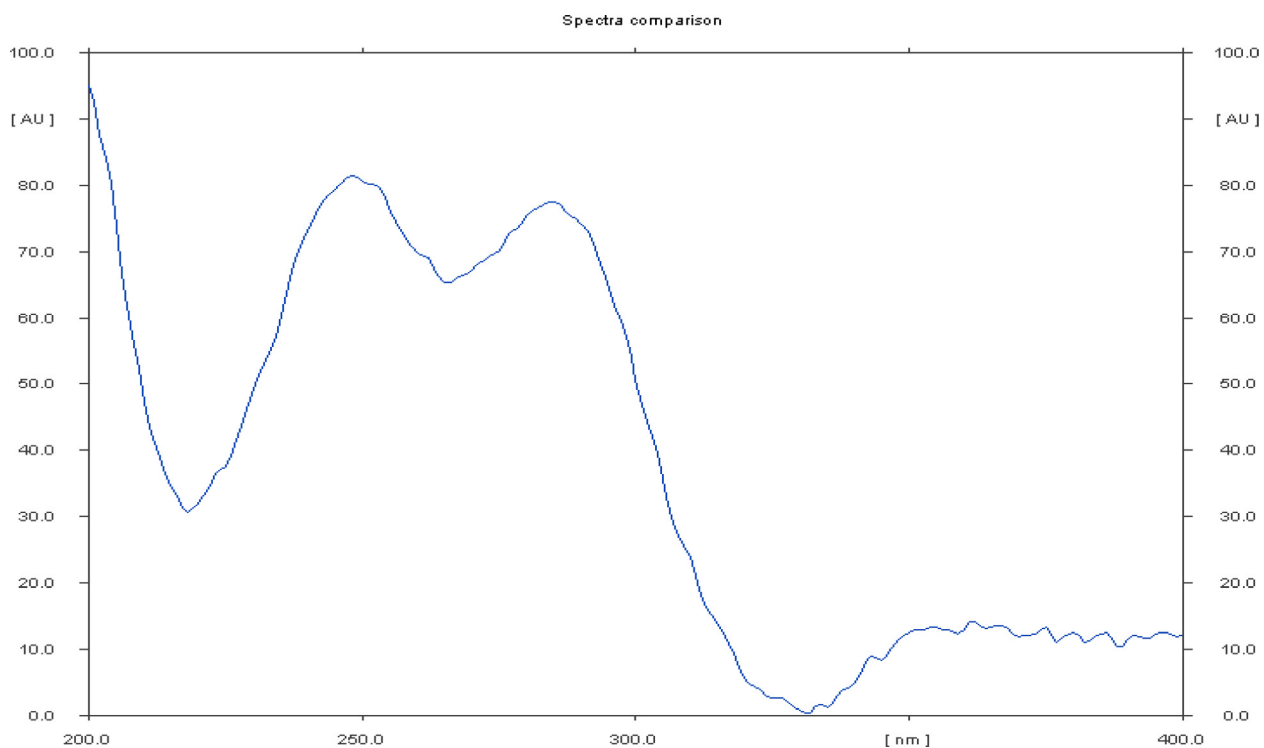


Fig. 6. Spectra of Peak observed at Rf Value 0.61.

extract different areas of the entire plant to establish a compounded sample eliminating possible constituents that may otherwise be present in this *Boswellia* species. In regards to HPTLC experimentation, further observations to establish standards for comparison of data is imperative to completely identify and confirm relevant phytochemical species obtained from preliminary test. Furthermore, HPTLC experimentation of better yields for natural samples as well more efficient separation of phytochemical constituents.

4. Conclusions

Natural medicinal and pharmaceutical materials became more apparent as there is a need for new innovative treatments and therapies for evolving illnesses. Phytochemical constituents are useful components needed to combat these illnesses as they exhibit antibacterial, anticancer, and antiviral effects. While many plants for example *Boswellia* species display these similar properties, much information is needed to identify their

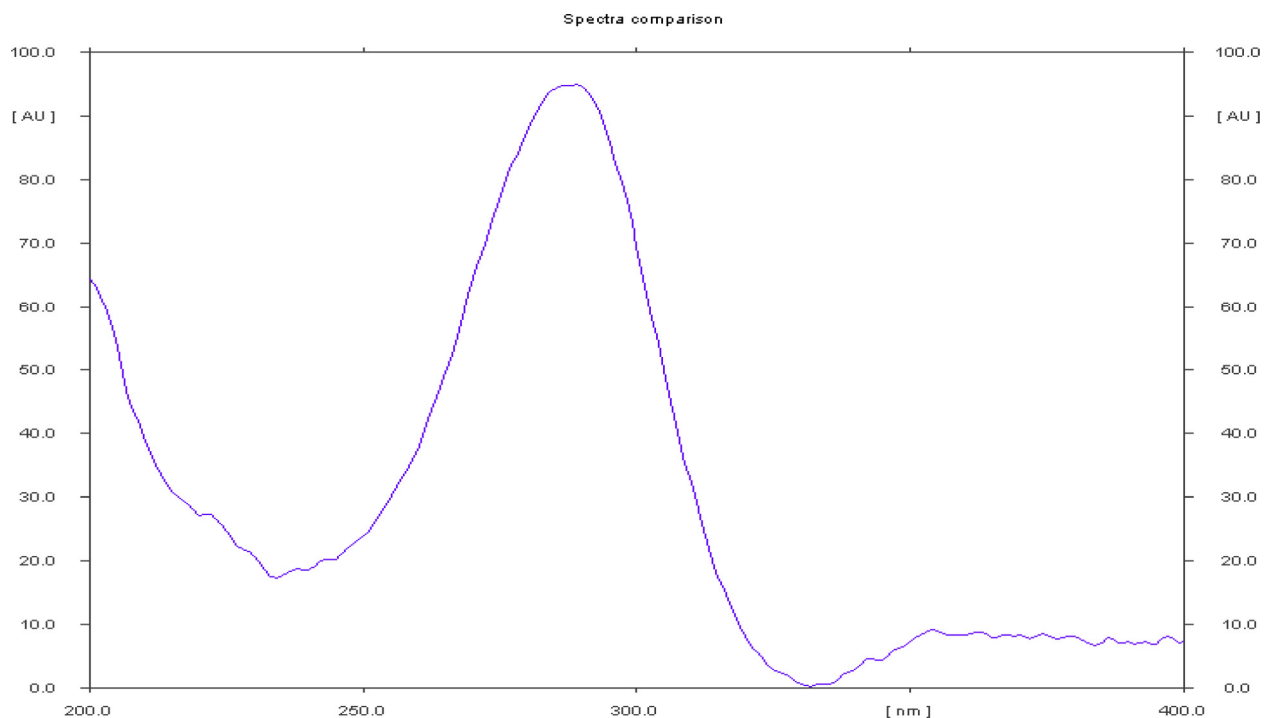


Fig. 7. Spectra of Peak observed at Rf Value 0.73.

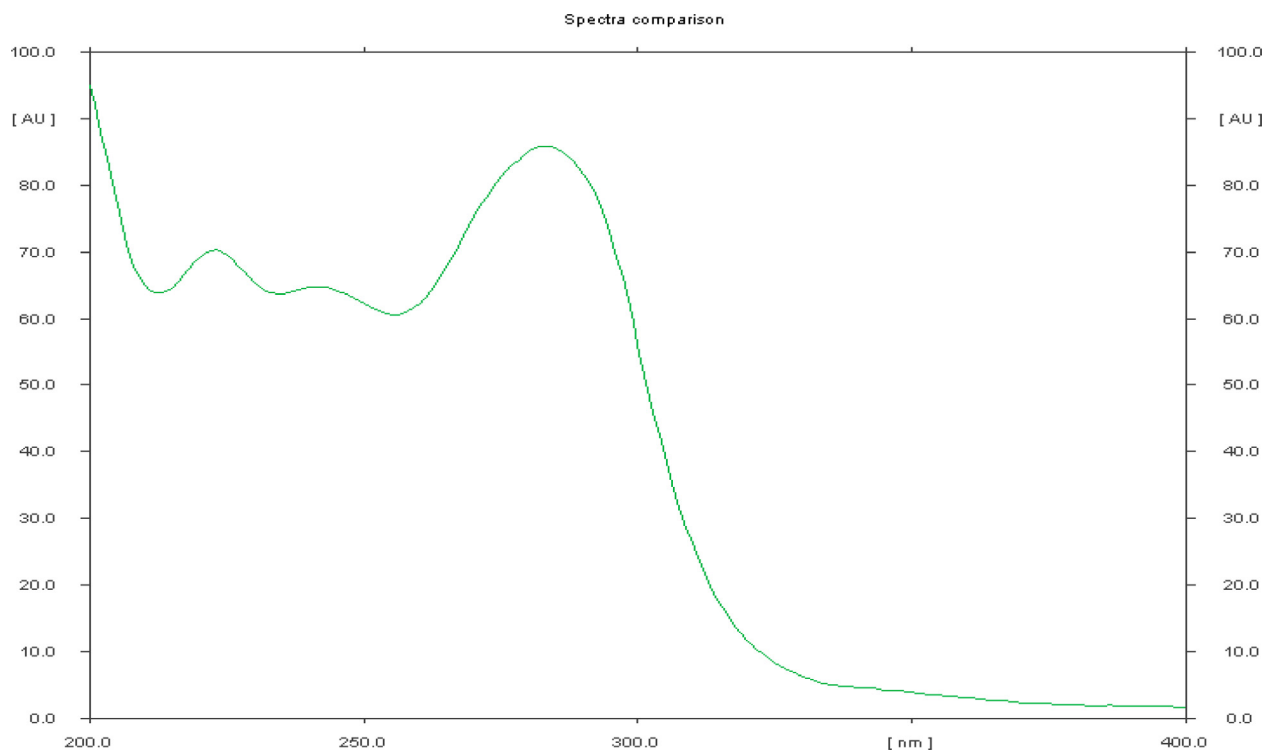


Fig. 8. Spectra of Peak observed at Rf Value 0.83.

chemical components. To identify these components, different analytical techniques are used. For the first time, a HPTLC finger print analysis of *Boswellia Sp. Burseraceae* was performed with preliminary phytochemical to determine the presence of several constituents via alkaloids, carbohydrates, and saponins. Moreover, this study will deem useful for establishing database for standard natural and herbal materials need for HPTLC analyses.

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Declaration of Competing Interest

The author declares no conflict of interest.

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