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Effects of Separation on the Phytochemical Properties and Antimicrobial Activity of Extracts and a Fraction of the African Mistletoe (*Loranthus micranthus* Linn) Leaves

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Abstract

The leaves of the African Mistletoe (*Loranthus micranthus* Linn) parasitic on *Kola acuminata* in Nsukka, Eastern Nigeria were studied. The crude petroleum ether (CPE) extract, ethanol extract of the CPE residue (EER) and ethylacetate residue fraction of CPE (C) were separated into bands using thin layer chromatography (TLC) technique. The CPE, EER and C chromatographic bands of the *Loranthus micranthus* Linn leaves were subjected to phytochemical and antimicrobial evaluation. Results of the phytochemical screening showed that the bands of CPE and EER afforded tannins, steroids and terpenes while that of C had tannins and terpenes only. Results of the antimicrobial activity revealed that CPE band 1 had an activity against *Staphylococcus aureus* while EER bands 1 and 2 were active against *Bacillus subtilis.* Band 1 of C alone was active against *Bacillus subtilis*, however, all the bands of C when combined had increased activity against other susceptible organisms suggesting synergism among the bands**.** The highest inhibition was noted in EER band 1 (20.30) while the least inhibition was observed in C band 2. Some of the band fractions showed lower activity while some showed similar level of activity when compared with the extract. The minimum inhibitory concentration (MIC) of the bands and that of the gentamycin ranged from 0.005 µg/ml – 0.2791 µg/ml.

Keywords: Chromatographic band fractions, thin layer chromatography, antimicrobial activity, phytochemicals and separation.

1. Introduction

The use of herbal medicine in the treatment of infection with microorganisms predates the introduction of antibiotics (Owoyale et al., 2005). The dependence on plant-sourced medicine apparently diminished with the advent of orthodox medication. However, the emergence of hitherto unknown disease causing microbes necessitates further drug research and development from any viable source including indigenous plants (Obasi et al., 2011). Furthermore, the possible benefits from alternative medical practice, especially in the developing parts of the globe, were limited by lack of scientific basis, warranting that the quality and consistency of the alternative medicines be ascertained and maintained for their maximal use and efficacy (Ukoha et al., 2011).

Extraction is the first crucial step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation steps ranges from pre-washing, drying of plant materials, grinding to obtain a homogenous sample (often by improving the kinetics of analytic extraction) to increasing the contact of sample surface with the solvent system. Care must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses (Fabricant and Farnsworth, 2001), then it is needed to prepare the extract as described by the traditional healer in order to mimic possibly the traditional 'herbal' drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll (Cos et al., 2006). It is very important to consider the polarity (non-polar to polar and thermally labile) of the compounds as to know the methods of extraction to be considered. Extraction methods, such as sonification, heating under reflux, soxhlet extraction and others are commonly used (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001) for the plant samples extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

Plant extracts are normally separated using any of the appropriate separation techniques to know whether better activity lies either in the crude extract or pure compounds. These extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the tremendous availability of chemical diversity (Cos et al., 2006). Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert

backing (Touchstone, 1992). It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent (Fair and Kormos, 2008). By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted. Also, the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column. As the chemicals being separated may be colourless, several methods exist to visualize the spots. Often a small amount of a fluorescent compound, usually manganese-activated zinc silicate, is added to the adsorbent that allows the visualization of spots under a blacklight (UV_{254}). The adsorbent layer will thus fluoresce light green by itself, but spots of analyte quench this fluorescence. Iodine vapours are a general unspecific colour reagent while specific colour reagents exist into which the TLC plate is dipped or which are sprayed onto the plate (TLC stains, 2013). Other reagents such bromine, Iodine and potassium permanganate are equally used in visualization of spots in TLC. Once visible, the R_f value, or retardation factor, of each spot can be determined by dividing the distance the product traveled by the distance the solvent front traveled using the initial spotting site as reference. These values depend on the solvent used and the type of TLC plate and are not physical constants. In organic chemistry, reactions are qualitatively monitored with TLC.

Loranthus micranthus is a semi parasitic shrub that grows on host trees. In ojoto and other Igbo speaking parts of Eastern Nigeria, it is called '*nbu nnunu*' literary meaning 'carried by bird' in apparent recognition of the role played by birds in dispersing the shrub. The host trees on which *L. micranthus* could grow include, *Kola acuminata, Kola nitida, Mangifera indica, Azadirachta indica, Jatropha curcas* and *Persia* sp. (Osadebe and Ukwueze, 2004). In Nigeria and other parts of Africa, the plant is used in ethno medication against diabetes and hypertension, schizophrenia and as immune booster (Osadebe et al., 2004; Osadebe and Omeje, 2009). Furthermore, reports of hypotensive potential of *L. micranthus* abound (Obatomi et al., 1996; Osadebe and Omeje, 2009; Ameer et al., 2010).

Crude petroleum ether (CPE) extract of *L. micranthus* contain tannins, terpenoids, flavonoids, steroid and saponins phytoconstituents, which have been implicated for the various antibacterial properties (Nwankwo and Egbuonu, 2011). *CPE was active against S. aureus, S paratyphyi, B. subtilis and K. spp.* The chloroform (A), ethyl acetate (B) and ethyl acetate residue (C) fractions of crude petroleum ether extract of *L. micranthus* Linn contain protein, flavonoids, steroids and terpenes,tannins (Egbuonu and Nwankwo, 2012). Among these fractions, C was active against the highest number of organisms which include; *Pseudomonas aeruginosa, B. subtilis and Klebsiella spp*.) (Egbuonu and Nwankwo, 2012)**.**

The distribution of phytochemicals (anti nutrients) in a plant may affect the quality (Nzewi and Egbuonu, 2011) and possibly content of other phytochemicals. For instance, tannins may form insoluble complexes with proteins, thereby decreasing protein digestibility and quality (Uzoechina, 2007). Therefore, the activities of mistletoe may also be dependent on the type of solvent used for its extraction or fractionation.

The present study therefore, attempts to separate the solvent fractions of crude petroleum ether extract in relation to their antimicrobial activity on some common pathogens. The separated compounds are then further tested for activity to determine their interactions (synergistic, additive or antagonistic) effects against the pathogens.

2. Materials and Methods

The solvents used (petroleum ether and ethyl acetate) are Sigma-Aldrich® grade. Other chemicals and standard grades of silica gel (70 to 230 mesh) and reagents used were of certified grade and quality and were used without further purification.

2.1 Test Organisms

The test organisms were clinical isolates of patients attending University of Nigeria Medical Centre, Nsukka donated to the Department of Microbiology, University of Nigeria Nsukka, Nigeria and include bacteria (*Bacillus subtilis* and *Staphylococcus aureus*)

2.2 Collection and Preparation of Plant Material

Fresh leaves of *L. micranthus* were collected from *K. acuminata* in Nsukka, Enugu State, South Eastern Nigeria. The specimen was then identified by a taxonomist in Bioresources Conservation and Development Programme (BCDP) unit, University of Nigeria, Nsukka, air dried for about eight days and pulverized using grinding machine. A known amount (1000 g) of the resultant powdery specimen, was extracted with 4.0 L of petroleum ether by Soxhlet method to obtain the crude petroleum ether extract (CPE). The residue was macerated with ethanol to obtain the ethanol extract of the residue (EER). The extract was filtered separately with Whatman filter paper. The filtrate was concentrated with rotary evaporator. Ten grams of the dried CPE was fractionated sequentially with chloroform and ethylacetate to obtain fractions A and B, respectively while the ethylacetate

residue became fraction C. The fractions were then stored in a refrigerator until used for the study (phytochemical and antimicrobial screening).

2.3 Determination of TLC Solvent System for Extracts and Fraction

Thin layer chromatography (TLC) was used to resolve the extracts and fractions following a standard procedure (Touchstone, 1992). Clean glass plates (20 cm by 20 cm) were coated with silica gel G 60 to a thickness of 0.25 cm using Kenso CJK 520 spreader. The coated plates were air dried and activated in an oven for 1 h at 110^0C. The plates were then cooled at room temperature. The crude petroleum ether extract (CPE) was dissolved in petroleum ether while the ethanol extract of the residue (EER) and ethylacetate residue fraction (C) were dissolved in ethanol and ethylacetate respectively. The dissolved extracts and fraction were spotted at different points on a plate, maintaining the same distance from one edge to another. The plates were allowed to dry at room temperature. The development of the spotted plates was tried in different solvent systems. The solvent system was allowed to travel a predetermined distance of 15 cm from the origin. The number of spots eluted was counted and their R_f values calculated.

2.4 Phytochemical Screening

The phytochemical analysis of the CPE, EER and C chromatographic bands of *L. micranthus* Linn from *K. acuminata* was done using standard methods as described in Evans (Evans and Trease, 2000). Specifically, the fractions were screened for saponins, glycosides, proteins, steroids, reducing sugars, alkaloids, flavonoids, tannins, terpenes, carbohydrates and resins.

2.5 The Minimum Inhibitory Concentrations (MIC) Agar Dilution Assay

The minimum inhibitory concentration was determined by agar dilution method, described by Adeniyi *et al*. (1996) and Harbone (1998). Essentially, one two-fold serial dilution of the fractions were prepared in sterile distilled water and poured into separate sterilized Petri dishes. The concentration was selected based on the preliminary sensitivity tests on the microorganisms as has been used and reported by many authors (Cleidson et al., 2007; Esimone et al., 2003). Essentially, nutrient agar powder (28 g) was weighed out and dispersed in 1 L of deionized water. The mixture was allowed to stand for 10 min before swirling to mix properly. It was sterilized by autoclaving for about 15 min at 121°C, and cooled to 40°C. Then, 20 ml of molten nutrient agar Sabouraud dextrose agar (SDA) (OXOID, UK) (for fungi) was poured into the Petri dishes, swirled slowly and then allowed to set and dry. Each set of agar plate was streaked with the broth culture of bacteria (*B. subtilis*, *S. aureus*, *E. coli, S. paratyphyi, A. niger, Klebsiella* spp. and *P. aeruginosa*) and fungi (*A. niger* and *C. albicans*). The agar plates without extract or standard antibiotic (the negative control) and the plates containing 2.5 µg/ml of a standard antibiotic, gentamicin sulphate (GS) (the positive control) were also streaked with the micro organisms. The agar plates were incubated at 37°C for 24 h (for the bacteria) and at 25°C for 48 h (for the fungus). The inhibition zone diameter (IZD), the measure of activity, was consequently determined by plotting the square of the inhibition zone diameter (IZD2) against the log concentration of the extract and the MIC was calculated from the intercept on the log concentration axis.

3. Results and Discussion

Different solvent systems used for the trial thin layer chromatography (TLC) with their respective R_f values were recorded. Results of the trial TLC showed that CEH, RFE and NRF were the best suitable developing solvent systems for the separation of CPE, EER and C respectively. NRF and RFE afforded five bands each while CEH gave six bands (Table 1). Thin Layer Chromatography (TLC) is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture.

Results of the phytochemical screening on the TLC band fractions from C showed that none of the band fractions possessed alkaloids, glycosides, carbohydrates, reducing sugars, flavonoids, resins and saponins. All the bands had tannins. Steroids were detected in bands 3 and 4 while terpenes were detected in band fractions 3, 4 and 5 (Table 2).

It was observed that phytochemical screening on the TLC band fractions from CPE showed the presence of the following compound; alkaloid, glycosides, carbohydrates, reducing sugars, flavonoids, resins, saponins were not detected in all the band fractions. Meanwhile, steroids and terpenes were contained in all the band fractions. Band fractions 1 and 2 afforded tannins while band 4 only gave resins (Table 3).

When subjected to phytochemical screening TLC band fractions from EER showed that all the band fractions contained tannins. Band fraction 4 contained alkaloids, glycosides, carbohydrates and reducing sugar in addition. None of the band fractions contained flavonoids and resins. Steroids and terpenes were found in all the band fractions except 4. However, only band fraction 4 possessed the phytochemical saponin (Table 4).

These phytochemicals confer these extracts and fraction with therapeutic activities (Rabe, 2000). It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). There is high tannin concentration in all the band fractions of CPE, EER and C. Tannins may have antioxidant activity because of their capacity to scavenge radicals (Santos and

Mello, 2004). In consonance with this, Bors *et al*. (2000) demonstrated the antioxidant activity of proanthocyanidins and hydrolysable tannins in red wine and green tea. According to Gupta (Gupta et al., 2002) saponin is contained in our EER band fraction 4 is an antioxidant. Terpenes, which were found to be present in some band fractions, have equally been implicated in anti-microbial activities (Rabe, 2000). None of the band fractions contained alkaloids, glycosides, carbohydrates, reducing sugars, flavonoids and resins except band fraction 4 of EER. *Loranthus micranthus* may therefore not be a good source of energy for humans. Various types of alkaloids have been isolated and used as antimalarials (Rosenthal and Goldsmith, 2001) but since none these fractions contained alkaloids, it means that they cannot be used against plasmodial activities. Flavonoids are another group of compounds with antioxidant activity.

Band fractions from C contained more of tannins than any other phytochemicals thus showing antioxidant potential of the fractions. The only EER band fraction with alkaloids, glycosides, carbohydrates and reducing sugar was fraction 4 which means that the fraction possessed both medicinal and nutritive properties. The other fractions of EER contained mostly tannins, followed by steroids and terpenes and finally saponins. Terpenes were abundantly found in all the fractions from CPE while trace amounts of steroids were found to be in them. Fractions 1 and 2 contained tannins while the only fraction with resins was 4.

At the concentration of 2.5 mg/ml of the TLC band fractions, antimicrobial activities were determined as recorded in table 5. Each of the extracts and C band fractions were tested against some gram positive bacteria (*Staph. aureus and B. subtilis*) because of their susceptibility than the gram negative bacteria (Gould and Booker, 2000).

The band fractions 1 and 2 of EER had inhibition zone diameters (IZDs) of 20.30 and 14.70 respectively while the IZD of the EER according to our previous study (Nwankwo and Egbuonu, 2011) was 20.00 against the organism *Bacillus subtilis*. The band fraction 1 which contains tannins, steroids and terpenes exhibited almost the level of inhibition when compared with the extract. Band fraction 2 with the same constituent compounds showed lower activity while band fractions 3, 4, 5 and 6 lost their activities upon separation due to dilution effect. This could be due to increased antagonism between the constituent compounds within the band fractions.

Band fraction 1 of CPE (5.30), followed by band 2 (23.70) had lower activity when compared with the extract (25.00) against *Staphylococcus aureus*. However, band fractions 2, 3, and 4 lost their activities when separated which suggests another dilution effect increased antagonistic effect. Most of the band fractions of CPE and EER lost activity and this is supported by Harbone (Harbone, 1984) who reported that the activity of plant extracts can sometimes change after fractionation and a pure crystalline compound may eventually be obtained which lacks the activity of the original extract.

Only band fraction 5 of fraction C lost its activity in the course of separation. Others which include bands 1, 2, 3 and 4 with the IZDs 3.00, 2.30, 3.70, and 4.70 respectively equally showed lowered activity but when combined showed additive effect with fraction C. Fraction C had an inhibition zone diameter of 20.00.

The minimum inhibitory concentration (MIC) tests of some active chromatographic band fractions and the standard drug were determined as outlined in table 6. The MIC of CPE band 5 (0.0558) which is less than 0.0625 µg/ml suggests that small amount of the drug is required to inhibit the growth of the susceptible organism *Staphylococcus aureus*. The chromatographic bands 1 and 2 of EER with the MICs of 0.2190 and 0.2791 respectively will require higher concentrations against the organism *Bacillus subtilis*. The minimum inhibitory concentration (MIC) of Fraction C bands combined to inhibit the growth of *Bacillus subtilis* is 0.2192. The standard drug gentamycin (0.005) suggests that this drug is very potent at a very small concentration against *Bacillus subtilis*.

4. Conclusion

Since it has been established that both the extracts, fractions and separated bands of *Loranthus micranthus* Linn possess antimicrobial activity, any of them can serve as therapeutic agent in the treatment of various diseases. However, some of the separated bands showed lower activity while some showed almost the same activity with the extract with fewer phytocompounds. The ones that showed similar level of activity with few phytochemicals should be considered most due to the low toxicity effect conferred on them by fewer compounds.

Attempt is being made to further purify the fractions with promising activity and this will in no small measure add to the development of new drugs used as pharmaceutical preparations and to the scientific world at large.

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References

Adeniyi, B., Odelola, H. & Oso, B. (1996), "Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae)". *African Journal of Medical Science* **25**,221-224.

Ameer, O., Salman, I. & Siddiqui, M. (2010), "Cardiovascular activity of the n-butanol fraction of the methanol extract of *Loranthus ferrugineus* Roxb", *The Brazilian Journal of Medical and Biological Research* **43**(2),186-194.

Bors, W., Michel, C. & Stettmaler, K. (2000), "Electron paramagnetic ressonance studies of radical species of proanthocyanidins and gallate esters". *Archive of Biochemical Biophysics* **2** (374), 347-355.

Cleidson, V., Simone, M., Elza, F. & Artur, S. (2007), "Screening Methods to Determine Anti-bacterial Activity of Natural Products", *Brazilian Journal of Microbiology* **38**, 369-380.

Cos, P., Vlietinck, A., Berghe, D. & Maes, L. (2006), "Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'". *Journal of Ethnopharmacology* **106**, 290–302.

Egbuonu, A. & Nwankwo, N. (2012), "Phytochemical properties of some solvent fractions of petroleum ether extract of the African mistletoe (*Loranthus micranthus* Linn) leaves and their antimicrobial activity", *African Journal of Biotechnology* **11**(62), 12595-12599.

Esimone, C., Ebebe, I., Chah, K. & Onyeka, C. (2003), "Comparative anti-bacterial effects of *Psidium guajava* aqeous extract", *Journal of Tropical Medicinal Plants* **4**(2),185-189.

Evans, W. & Trease, E. (2000), *Pharmacology*. 14th Edn., WB Saunders Company Ltd. pp.224-293.

Fabricant, D. & Farnsworth, N. (2001), "The value of plants used in traditional medicine for drug discovery", *Environment and Health Perspectives* **109**, 69–75.

Fair, J, & Kormos, C. (2003), "Practice of chromatography", *Journal of Chromatography* **121**(1-2), 49-54.

Gould D, Booker C. (2000), *Applied microbiology for nurses*. Aardvark Editorial, Mcndharn, Suffork, pp:75-94

Gupta, S., Prakash, J. & Srivastava, S. (2002), "Validation of traditional claim of *Tulsi ocimun sanctun.* Linn as a medicinal plant", *Indian Journal of Experimental Biology* **40**, 765-773.

Harbone, J. (1984), *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. (2nd edn). Chapman and Hall, London, pp. 1, 19, 37 – 168.

Harbone, J. (1998), *Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis*. Chapman, Hall, London, pp.182-190.

Nwankwo, N. & Egbuonu, A. (2011), "Phytochemical and antimicrobial activity of petroleum ether extract of the African Mistletoe (*Loranthus micranthus* Linn) leaves", *International Research Journal of Pharmacy and Pharmacology* **1**(8), 211-214.

Nzewi, D. & Egbuonu, A. (2011), "Effect of boiling and roasting on the proximate properties of asparagus bean (*Vigna Sesquipedalis*). *African Journal of Biotechnology* **10**(54),11239-11244

Obasi, N., Ejikeme, P. & Egbuonu, A. (2011), "Antimicrobial and phytochemical activity of methanolic extract and its fractions of *Jatropha curcas* Linn. (Eurphorbiaceae) stem bark", *African Journal of Pure and Applied Chemistry* **5**(5), 92-96.

Obatomi, D., Aina, V. & Temple, V. (1996), "Effects of African Mistletoe on Blood Pressure in Spontaneously Hypertensive Rats". *International Journal of Pharmacognosy* **34**(2), 124-127.

Okwu, D. (2001), "Evaluation of the chemical composition of indigenous spices and flavouring Agents", *Global Journal of Pure and Applied Science* **7**(3), 455- 459.

Osadebe, P., Okide, G. & Akabogu, I (2004), "Study on anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn) sourced from five different host trees", *Journal of Ethnopharmacology* **95**,133-138.

Osadebe, P. & Omeje, E. (2009), "Comparative acute toxicities and immunomodulatory potentials of five Eastern Nigeria Mistletoes", *Journal of Ethnopharmacology* **126**(2), 287-293.

Osadebe, P. & Ukwueze, S. (2004), "A comparative study of the phytochemical and antimicrobial properties of the Eastern Nigerian species of African Mistletoe (*Loranthus micranthus*) sourced from different host areas trees", *Journal of Biological Research and Biotechnology* **2**(1),18-23.

Owoyale, J., Olatunji, G. & Oguntoye, S. (2005), "Antifungal and Antibacterial Activities of an Alcoholic Extract of *Senna alata* leaves", *J. Appl. Sci. Environ*. **9**(3),105-107.

Pharmacopoeia of the People's Republic of China (2000). English Ed. The Pharmacopeia Commission of PRC,Beijing.

Rabe, T. (2000), "Isolation of antimicrobial sesquiterpenoid from *Warbugie salutarius*. J. *sanctun* . Linn as a medicinal plant", *Indian Journal of Experimental Biology* **40**, 765-773.

Rosenthal, P. & Goldsmith, R. (2001), *Antiprotozoal Drugs*. In Basic and Clinical Pharmacology, 8th edition. McGraw-Hill Companies Inc.

Santos, S. & Mello, J. (2004), *Farmacognosia da planta ao medicamento*. 5.ed. Porto Alegre/Florianópolis: Editora da URFGS/UFSC, pp.615-656.

The Japanese Pharmacopeia (2001), Fourteenth ed., JP XIII, *The Society of Japanese Pharmacopeia*, Japan.
Thin Layer Chromatography stains http://www.reachdevices.com/TLC_stains.html. Accessed on 23rd of March, 2013.
Touchstone, J. (1992), *Practice of Thin-layer chromatography*, John Wiley and Sons, Ave. New York, pp. 264 – 265.
Ukoha, P., Egbuonu, A., Obasi, N. & Ejikeme, P. (2011), "Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities", *African Journal of Pure and Applied Chemistry* **5**(8), 237-244.
United States Pharmacopeia and National Formulary (2002), USP 25, NF 19. United States Pharmacopeial Convention Inc., Rockville.
Uzoechina, O. (2007), "Evaluation of the effect of processing techniques on the nutrient and antinutrient contents of Pigeon Pea (*Cajanus cajan*) seed flours", Journal of Food Science **28**, 76-77.

TABLE 1**:** DIFFERENT SOLVENT SYSTEMS USED FOR THE TRIAL TLC

Extracts/Fraction	**Different solvent systems and R_f values of the spots**						
	HOT	**DEH**	**CEH**	**XDM**	**RFE**	**NRF**	**ETM**
CPE	0.56 0.47 0.18	0.80 0.70 0.65	1.05 0.47 0.17	1.05 0.90	0.88 0.47 0.28 0.20 0.09	0.75 0.65 0.60	0.85 0.75 0.70
EER	0.69 0.38	0.30 0.25 0.20	0.86 0.78 0.65 0.51 0.24 0.07	0.80 0.65 0.53	0.55 0.50 0.48	0.40 0.36 0.30 0.32	0.30 0.25 0.17
C	0.69 0.57 0.40	1.00 0.47 0.19	0.79 0.19	0.21 0.19	0.79 0.76 0.65	0.94 0.91 0.83 0.80 0.50	0.15 0.12

HOT = Hexane: toluene: methanol (6: 4: 2)
DEH = Dichloromethane: petroleum ether (4: 6)
CEH= Chloroform: petroleum ether (10: 1)
XDM = Dichloromethane: Hexane (2: 6)
RFE = Chloroform: petroleum ether (2: 1)
NRF = Chloroform: methanol: ethylacetate (1: 4: 2)
ETM = Ethylacetate: toluene: methanol (2: 4: 8)

TABLE 2: PHYTOCHEMICAL ANALYSIS OF THE CHROMATOGRAPHIC BANDS FROM C

Parameter	BF 1	BF 2	BF 3	BF 4	BF 5
Alkaloids	-	-	-	-	-
Glycosides	-	-	-	-	-
Carbohydrates	-	-	-	-	-
Reducing Sugars	-	-	-	-	-
Flavonoids	-	-	-	-	-
Resins	-	-	-	-	-
Tannins	++	++	+++	+++	+
Steroids	-	-	+	+	-
Saponins	-	-	-	-	-
Terpenes	-	-	+	+	+

+ = Present; - = absent; multiple pluses indicate the degree of abundance.
BF = band fraction

TABLE 3: PHYTOCHEMICAL ANALYSIS OF CHROMATOGRAPHIC BANDS FROM CPE

Parameter	BF 1	BF 2	BF 3	BF 4	BF 5
Alkaloids	-	-	-	-	-
Glycosides	-	-	-	-	-
Carbohydrates	-	-	-	-	-
Reducing Sugars	-	-	-	-	-
Flavonoids	-	-	-	-	-
Resins	-	-	-	++	-
Tannins	+++	+++	-	-	-
Steroids	+	+	+	+	+
Saponins	-	-	-	-	-
Terpenes	+++	+	++	+	+ ++

+ = Present; - = absent; multiple pluses indicate the degree of abundance.
BF = band fraction

TABLE 4: PHYTOCHEMICAL ANALYSIS OF CHROMATOGRAPHIC BANDS FROM EER

Parameter	BF 1	BF 2	BF 3	BF 4	BF 5	BF 6
Alkaloids	-	-	-	++	-	-
Glycosides	-	-	-	++	-	-
Carbohydrates	-	-	-	+	-	-
Reducing Sugars	-	-	-	+	-	-
Flavonoids	-	-	-	-	-	-
Resins	-	-	-	-	-	-
Tannins	+++	+ ++	+++	+	+++	+++
Steroids	+ +	+	+	-	+	+
Saponins	-	-	-	++	-	-
Terpenes	++	++	+	-	++	+

+ = Present; - = absent; multiple pluses indicate the degree of abundance.

TABLE 5: ANTIMICROBIAL SCREENING TEST OF THE TLC BAND FRACTIONS AT THE CONCENTRATION OF 2.5MG/ML

BF	EER Mean IZD (mm)	Organism
1	20.30	*B. subtilis*
2	14.70	*B. subtilis*
3	-	*B. subtilis*
4	-	*B. subtilis*
5	-	*B. subtilis*
6	-	*B. subtilis*
	CPE	
1	5.30	*Staph. aureus*
2	-	*Staph. aureus*
3	-	*Staph. aureus*
4	-	*Staph. aureus*
5	23.70	*Staph. aureus*
	Fraction C	
1	3.00	*B. subtilis*
2	2.30	*B. subtilis*
3	3.70	*B. subtilis*
4	4.70	*B. subtilis*
5	-	*B. subtilis*
1, 2, 3 & 4 combined	20.00	*B. subtilis*

Results are expressed as simple mean of two tests.
— = No inhibition zone; IZD = Inhibition zone diameter.

TABLE 6: MINIMUM INHIBITORY CONCENTRATION (MIC) TEST OF SOME ACTIVE CHROMATOGRAPHIC BAND FRACTIONS AND THE STANDARD DRUG GENTAMYCIN

Bands	MIC (mg/ml)	Organism
CPE Band 5	0.0558	*Staph. aureus*
EER Band 1	0.2190	*B. subtilis*
EER Band 2	0.2791	*B. subtilis*
C Bands 1, 2, 3 & 4 combined	0.2192	*B. subtilis*
Gentamycin	0.005	*B. subtilis*

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