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Research Article

Extraction and Characterisation of Phytochemicals from White Seringa (*Kirkia acuminata*) Bark Extracts

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Abstract

The aim of the study was to extract and characterise phytochemicals which were believed to possess analgesic effect from *Kirkia acuminata* bark extracts. Soxhlet extraction and Steam distillation methods were used for the extraction of compounds. Methanol, dichloromethane and hexane were used as extraction solvents. Classes of phytochemicals were identified by qualitative tests and Thin Layer Chromatography (TLC) using UV light. The qualitative tests for the phytochemical screening indicated the presence of many compounds including alkaloids, anthraquinones, glycosides, flavonoids, phenols, tannins. Alkaloids, flavonoids, phenols and tannins were observed on TLC. Menthol, catechol, 1,2 benzenediol 4 methyl, nitro phenyl salicylate, phenol dimethoxy, tau-cadinol, Isopropyl l,8 dimethyl, menthone and levomenthone were identified using Gas Chromatography-Mass Spectrometer. The polar solvents showed compatibility with the various chemical classes. The presence of these compounds gave *Kirkia acuminata* its characteristic property of being an analgesic.

Keywords: Kirkia acuminata; Phytochemicals; Analgesic; Thin Layer Chromatography; Gas Chromatography-Mass Spectrometer

Introduction

Kirkia acuminata belong to the Kirkiaceae family. It is found as a planted exotic tree at the University of Zimbabwe, Harare and in several cities in Zimbabwe. Its common names are Mubvumira, Mutsakatidze and Mutuhwa (Shona), Umvumile (Ndebele) and White seringa (English). It is found all over African Countries e.g. South Africa, Botswana, Namibia, Mozambique, Malawi, Zimbabwe and Tanzania. *Kirkia acuminata* is a drought resistant plant, which grows very well in hot and dry areas, and it is quite sensitive to frost [1]. It may be found on various soil types from fertile soils (alluvial flats), sandy or loamy soils near rivers to sandy, dry soils and rocky slopes, although well-drained, basic soils are more preferred [2,3]. It reproduces itself using seed or stem parts and it is fast growing. The plant has got different uses but in Africa it is mainly known for its medicinal uses. An infusion of the bark is taken against vomiting and abdominal pains, infusion of the root is taken to treat coughs [4,5]. The fruit sap is applied on wounds and as an antidote to snake bites. Pulverized roots are a remedy for toothache [5,6].

According to the World Health Organisation, a medicinal plant is any plant whose parts contains substances that can be used for healing purposes, or which are used during the chemo-pharmaceutical semi synthesis [7,8]. The plant parts including roots, leaves, rhizomes, stems, barks, flowers, fruits, grains or seeds, are the ones used in the control or treatment of a disease condition and they contain some chemical components or substances that have medicinal effects. These chemical substances from plants are commonly known as phytochemicals. These chemical substances are responsible for acting against microbial infections or attack by pests [9-11].

Alkaloids are considered as one of the largest and well known groups of secondary metabolites. Alkaloids are made from ammonia compounds consisting of nitrogen bases prepared from amino acid blocks [12]. Glycosides are produced from the condensation products of sugars. Glycosides are classified basing on the type of sugar component, chemical nature of aglycone as well as their pharmacological action [13,14].

A plant that is selected basing on the traditional uses should be prepared in a way described by the herbalist in order to produce as closely as possible the traditional medicine [15,16]. The nature of the bioactive compound under investigation has got an impact on the selection of the solvent system to be used. Various solvent medium are available for extraction of phytochemicals from plant samples.

Materials and Methods

Materials

Methanol, hexane, dichloromethane, acetonitrile both HPLC grade, and de-ionised water were used for the research.

Preparation of Kirkia acuminate barks

The *Kirkia acuminata* barks were air dried in the laboratory over a period of two weeks. The barks were then ground into fine powder with a pulveriser which was provided by Institute of Mining Research (IMR) at the University of Zimbabwe.

Extraction experiments

Soxhlet Extraction

The fine powder was divided into three 50g samples labelled A, B and C respectively. These were then extracted separately using 150 ml of a specific solvent. Sample A was extracted with hexane, Sample B with dichloromethane and sample C was extracted with methanol respectively. The extractions were allowed to run for 6 hours. After extraction, the samples were evaporated on the rotary evaporator to remain with important ingredients. The samples were then taken for analysis on the Gas Chromatography-Mass Spectrometer (GC-MS).

Steam Distillation Experiment

A mass of 200g sample was weighed and placed in a 1000 ml round bottomed flask and 500 ml of water was added. It was steam distilled for 8 hours. The distillate was extracted with hexane, followed by dichloromethane by liquid-liquid solvent extraction using three 200 ml portions in each extraction. To separate the oil from the solvents, hexane fraction and dichloromethane was evaporated on the rotary vapour. The oil obtained was analysed by GC-MS. Phytochemical Screening of secondary metabolites was done using chromatographic separation methods.

Results

Secondary metabolite	Name of test	Methanol Extract	Dichloromethane extract	Hexane extract	Essential Oils
Alkaloid	Wagner test	+	+	-	+
Anthraquinone	Born Trager's test	+	+	-	+
Cardiac glycosides	Kellar Kiliani Test	-	-	-	-
Flavonoid	NaOH test	+	-	-	+
Phlobatannin	Test	-	-	-	-
Phenol	Ferric chloride test	+	+	+	+
Reducing sugar	Fehling test	+	+	-	-
Saponin	Frothing test/Foam test	+	+	-	-
Steroid	Liebermann-Burchardt test	+	+	-	+
Tannin	Braemer's test	+	+	-	+
Terpenoid	Salkowski test	+	+	-	+
Volatile oil	-	-	-	-	-
Proteins and amino acids	Xanthaproteic test	-	-	-	-

Table 1: Phytochemical screening assay.

Key:

+: Indicates presence of compound under investigation

-: Indicates absence of compound under investigation

	Alkaloid (orange)	Tannins (brownish grey)	Phenol (green)	Flavonoids (orange)
Methanol	+	+	+	+
Hexane	-	-	+	-
Dichloro-methane	+	+	-	-
Essential Oils	+	+	+	+

Table 2: Results for Thin Layer Chromatography.

Key: Colours written indicates the expected colour for a positive result.

Compound name	Retention Time/ minutes	Quantity of compound in 50 g sample	% of compound in sample
Menthol	2,85	5,70	11,40
Catechol	2,88	5,76	11,52
1,2 benzenediol-4 methyl	2,94	5,88	11,76
Nitro phenyl salicylate	13,31	0,27	0,54
Phenol dimethoxy	13,67	2,40	4,80
Tau-cadinol	17,39	2,97	5,94

Table 3: Methanolic Extracts.

Key: % of compound in sample = (quantity of compound in 50g sample/50g)*100

Compound Name	Retention Time/ minutes	Quantity of the compound in 50g sample	% of compound in sample
Catechol	11,52	2,88	5,76
1,2 benzenediol 4-methyl	12,85	2,88	5,76
Isopropenyl,8 dimethyl	19,35	2,70	5,40

Table 4: Dichloromethane extracts.

Compound Name	Retention Time/ minutes	Quantity of compound in 50g sample	% age of compound in sample
Catechol	11,57	2,79	5,58
1,2 benzenediol -4 methyl	12,90	2,61	5,22

Table 5: Hexane extracts.

Compound Name	Retention Time/ minutes	Quantity of compound in 200g sample	% of compound in sample
Menthone	10,88	2,45	1,23
Levomenthol	11,14	2,25	1,13
Catechol	11,55	2,25	1,13
1,2 benzenediol, 4 methyl	12,86	2,40	1,20
Tau-cadinol	17,57	2,38	1,19

Table 6: Steam distillation extracts (essential oils).

Discussion

Tables 3, 4 and 5 show results of *Kirkia acuminata* barks extraction using solvents of different polarities including methanol, dichloromethane and hexane respectively, table 6 shows extraction of essential oils by steam distillation. Methanol was acting as the polar solvent, dichloromethane medium polar and hexane as the non-polar solvent. The extracts were subjected to three different tests; phytochemical screening by qualitative tests, TLC analysis and GC-MS respectively.

Table 1 results showed that non-polar solvent hexane had a low compatibility with most of the phytochemicals. This was shown by the negative results obtained from the qualitative tests, only phenols were found to be present out of all the phytochemicals that were tested using hexane extract. This showed that phytochemicals found in *Kirkia acuminata* are polar compounds. A polar solvent (methanol) and a medium polar solvent (dichloromethane) dissolved most of the phytochemicals in the plant barks. This was indicated by the positive results from the analysis performed by the qualitative tests. Essential oils extracted by steam distillation on table 6 showed the presence of most phytochemicals that were tested for. These results showed that phenol was present in all the four extracts. Glycosides were absent in all the extracts. Saponins and reducing sugars were only found in the methanol and dichloromethane extracts shown in tables 3 and 4 respectively. Flavonoids were present in methanol extract and essential oils only. Alkaloids, anthraquinones, steroids, tannins and terpenoids were found to be present in other extracts except for the hexane extract. The aqueous extract which is typically used by the traditional healers for treatment of various ailments showed presence of practically 70% of the tested phytochemicals. The results from qualitative tests gave information on different classes of phytochemicals that are present in the plant barks. These classes account for different activities: analgesic, antipyretic, antimicrobial, antispasmodic, etc. *Kirkia acuminata* thus has a broad spectrum of medicinal related activities attributed to the presence of all these phytochemicals.

Table 2 on TLC shows that only coloured compounds were observed. The compounds were observed under UV light and then compared with the colours for phytochemicals found in literature. Four classes of phytochemicals were observed on the TLC plates for methanolic extracts and essential oils from steam distillation. These observed phytochemicals were alkaloids (orange), tannins (brownish grey), phenols (green) and flavonoids (orange). The coloured compounds observed using TLC also gave positive results when tested by qualitative analysis. When TLC plate for the hexane extract was visualised, a green spot was observed showing the presence of phenols. This proved to be correct since phenols also gave a positive result when tested by qualitative analysis. Two spots coloured orange and brownish-grey were observed when the TLC plate for the dichloromethane plate was visualised under UV light.

GC-MS identified a lot of compounds in each of the extracts but the compounds that were being investigated in this study are the ones with analgesic effects since the plant barks are mainly used for treating stomach pains [17-19]. The information obtained by the GC/MS was solely dependent on the NIST library in the system. The compounds with analgesic effects that were identified from the methanolic extract included menthol, catechol, 1, 2 benzenediol 4 methyl, nitro phenyl salicylate, phenol dimethoxy and tau-cadinol. Catechol, 1, 2 benzenediol 4 methyl and Isopropyl, 8 dimethyl were identified from dichloromethane extract. Compounds identified from the hexane extract were catechol and 1, 2 benzenediol, 4 methyl. Menthone, levomenthol, 1, 2 benzenediol, 4 methyl catechol and tau-cadinol were identified in essential oils. These compounds were identified at different retention times. The compounds account for analgesic activity of the plant *Kirkia acuminata*. The results from the study indicated that catechol was identified in all the extracts. Therefore catechol is the major component in the plant barks of *Kirkia acuminata*. Catechol is known as a compound that is formed from the hydroxylation of phenols and also it is a component of castoreum. Castoreum is used as an analgesic, anti-inflammatory and antipyretic [5,20]. This shows that catechol belongs to the class of phenols. 1, 2 benzenediol and 4 methyl were also identified in all the extracts. It is also known as 4 methyl catechol. So it is a methylated catechol therefore it also belongs to the class of phenols. Menthone, menthol and levomenthol were identified from methanol extracts and essential oils, these compounds belong to the class of terpenes (mono terpenes). They are known as topical analgesics, used to relieve minor aches and pains [10,19,21]. Isopropyl, 8 dimethyl was identified from the dichloromethane extract belonging to the terpenoids it's a sesquiterpenes. Cadinol was identified from the methanol extract and essential oils it is a sesquiterpenoid alcohol. It is mainly found in essential oils and extracts of many plants [22,23]. Nitro phenyl salicylate is also known as salol and it can be formed by heating salicylic acid with phenol. It has been used as an antiseptic based on the antibacterial activity upon hydrolysis in the small intestines and it acts as a mild analgesic [13,21]. From its synthesis this shows that salol has got phenolic properties as well as salicylates properties [17,24]. Therefore its presence in the barks has got some activities in analgesic effects.

The results indicated that some of the classes obtained from the phytochemical screening and TLC have other activities other than analgesic effect since the 56 compounds identified which belong to those classes do not have analgesic activities [6,11]. They might have other activities like repellent, larvicidal, antimicrobial, etc. However to a larger extent the findings demonstrated that essential oils and aqueous extracts from *Kirkia acuminata* bark extracts possess the analgesic activity [10,14,15] although they have a small percentages ranging from 0,54 to 5,88%.

It is known that the active constituents of medicinal plants are affected by many factors and may vary during the course of plant growth. Proper time of collection is very important to obtain a drug of good quality. Factors that are known to affect the presence or absence of active constituents in a plant are annual seasons, time of the day and stage of maturity and age when the plant parts are collected.

Conclusion

The present study indicated that for the qualitative and Thin Layer Chromatography analysis methanol and steam distillation have the highest compatibility with most of the phytochemicals followed by dichloromethane and hexane extractions.

Phytochemical screening results indicated the presence of alkaloids, anthraquinones, glycosides, flavonoids, phenols, tannins. Alkaloids, flavonoids, phenols and tannins were observed on Thin Layer Chromatography using UV Light.

Menthol, catechol, 1,2 benzenediol 4 methyl, nitro phenyl salicylate, phenol dimethoxy, tau-cadinol, Isopropenyl, 8 dimethyl, menthone and levomenthone were identified using Gas Chromatography-Mass Spectrometer.

The hexane fraction which is highly a non-polar solvent showed very few phytochemicals in it. The presence of these compounds suggest *Kirkia acuminata* possess analgesic properties.

Bibliography

1. Abebe H., *et al*. "Phytochemical Investigation on the Roots of Solanum, Incanum, Hadiya Zone". *Ethiopia Journal of Medicinal Plants Studies* 2.2 (2014): 354.

2. Cosa P., *et al*. "Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'". *Journal of Ethnopharmacology* 106.3 (2006): 290-302.

3. Prasad KB., *et al*. "Compositional characterization of traditional medicinal plants: Chemo-metric approach". *Archives of Applied Science Research* 2.5 (2010): 1-10.

4. Daffre S., *et al*. "Bioactive natural peptides". In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry 35 (2008): 597- 691.

5. Philipson JD. "Phytochemistry and pharamacognosy". *Phytochemistry* 68.22-24 (2007): 2960-2972.

6. Heywood VH., *et al*. "Flowering plant families of the world". Richmond Hill, Ontario, Firefly Books, Canada (2007): 102.

7. Deba F., *et al*. "Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from Bidens pilosa Linn. Var. Radiata". *Food Control* 19.4 (2008): 346-352.

8. Maurya R., *et al*. "Antiosteoporotic agents from Natural sources". In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry 35 (2008): 517-545.

9. Abo KA., *et al*. "Antimicrobial potential of Spondias mombin, Croton zambesicus and Zygotritonia crocea". *Journal of Pharmacological Research* 5 (1991): 494-497.

10. Liu RH. "Potential synergy of phytochemicals in cancer prevention: mechanism of Action". *Journal of Nutrition* 134.12 (2004): 3479-3485.

11. Doughari JH., *et al*. "Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria". *Journal of Medicinal Plants Research* 3.11 (2009): 839-848.

12. Derby C and Margotteaux G. "Relationship between essential fatty acids and tissue antioxidant levels in mice". *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales* 164.12 (1970): 2675-2681.

13. Kar A. "Pharmaocgnosy and Pharmacobiotechnology (Revised-Expanded Second Edition), New Age". International Limited Publishers New Delhi (2007): 332-600.

14. Firn R. "Nature's Chemicals". Oxford University Press, Oxford (2010): 74-75.

15. Eloff JN. "Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation". *Phytomedicine* 11.4 (2004): 370-371.

16. Oleszek W and Marston A. "Saponins in food and medicinal plants". Kluwer academic publishers, New York (2000): 1-95.

17. Martinez MJA., *et al*. "Anti-infectious activity in the anthemideae tribe". In: Atta-ur- (Ed.) Studies in Natural Products Chemistry 35 (2008): 445-516.

18. Mattson MP and Cheng A. "Neurohormetic phytochemicals: low dose toxins that induce adaptive neuronal stress responses". *Trends in Neurosciences* 29.11 (2006): 632-639.

19. Sarker SD and Nahar L. "Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. England, John Wiley and Sons (2007): 283-359.

20. Madziga HA., *et al*. "Phytochemical and Elemental Analysis of Acalypha wilkesiana Leaf". *Journal of American Science* 6.11 (2010): 510-514.

21. Sen CK. "Oxygen toxicity and antioxidants: state of the art". *Indian Journal of Physiology and Pharmacology* 39.3 (1995): 177-196.

22. Chen FW., *et al*. "Evaluation of the antioxidant activity of Ruellia tuberosa". *Food Chemistry* 94.1 (2006): 14-18.

23. Mehta JP. "Separation and Characterization of anthraquinone derivatives from Cassia Fistula using Chromatographic and Spectral Techniques". *International Journal of Chemical Sciences* 10.1 (2012): 306-316.

24. Masoko P. "Ethnobotanical study of some selected medicinal plants used by traditional healers in Limpopo Province (South Africa)". *American Journal of Research Communication* 1.8 (2008): 8-23.

25. D'Incalci M., *et al*. "Use of cancer chemopreventive phytochemicals as antineoplastic agents". *Lancet Oncology* 6.11 (2005): 899-904.

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