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Original Article

Extraction and Profiling of Phytochemicals Present in Selected Ornamental Plants in Kericho County

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ABSTRACT

Bacterial infections are prevalent in most parts of Kenya causing diseases like pneumonia, typhoid, cholera and meningitis. This has contributed to unsustainable socio-economic development following the emergence of antimicrobial resistant strains of bacteria and hence the need for alternative strategies that are not only effective against bacteria but environmentally safe as well. This study was designed to extract and profile phytochemicals present within crude leaves, flowers and roots extracts of Iris versicolor and Nerium oleander for future antimicrobial activities. The plant parts were macerated and extracted to obtain phytochemicals that were then identified under different classes of compounds by treating them with different reagents following standard laboratory procedures. About 500gms of the grounded powder of the parts were soaked in 1.5litres of Hexane, DCM and Methanol and left to settle for 24 hours after which the solutions were filtered with Whatman No. 1 filter paper. The filtrate was then evaporated to a paste using a rotary evaporator equipment. The paste was aliquoted into 20gms and refrigerated for future qualitative analysis. About 2mls of different test solutions were added to 1ml of the plant extracts solutions to obtain tannins, saponins, terpenoids, flavonoids, steroids, phenols and alkaloids. It was found that the test results of the three solvents used for the extraction of the phytochemicals showed gradient elution of the phytochemicals increasing from Hexane, DCM and Methanol. Hexane extracted tannins, flavonoids and phenols while DCM extracted tannins, saponins, flavonoids and alkaloids. Methanol with highest polarity extracted tannins, saponins, terpenoids, flavonoids and phenols. It was also observed that the polarity of the solvent determined the type of bioactive compounds with the most polar extracting different classes of phytochemicals than the less polar solvent. In all considerations, methanolic extract of Nerium oleander leaves and flowers showed positive results for all the phytochemicals present in selected plant extracts.

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INTRODUCTION

Ornamental plants have been known to have secondary metabolites that can be extracted and used to manage ailments (Selwal *et al*., 2023). In an ideal situation, we desire a good-looking environment and health; we also need to enjoy primary health care, the need for smooth skin, good hair and smooth face. Among the SDGs, there is need for good life on primary health care as one of the government's big four agenda. SDG 3 aims to prevent needless suffering from preventable diseases and premature death by focusing on key targets that boost the health of a country's overall population (Parmar, n.d.). This goal addresses all major health priorities, reproductive, maternal, newborn, child and adolescent health, communicable and non-communicable diseases, universal health coverage and access for all to safe, effective, quality and affordable medicines and vaccines. The desire for good health has been hindered by expensive drugs, and bacterial resistance due to synthetic drugs which has led to multi-drug resistance increasing microbial resistance thus the need to improve efficacy that deteriotes over time therefore continuous testing. In addition, the ornamentals knowledge is vital in the pharmacological application in that some of these phytochemicals have been thought to be responsible for the antimicrobial activity shown by the extracts from the two plants, this may provide an effective and alternative available antimicrobial agent for people who cannot afford conventionally synthetically produced medicine and therefore this study seeks to determine the phytochemical extracts from leaves, flowers and roots of *lris versicolor* and *Nerium oleander* and evaluate their antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli*. For this reason, these Phytochemicals are non-nutritive plant chemicals with protective or disease-preventive properties. They are non-essential nutrients, in the sense that they are not a strict requirement for sustaining life. (Uzochukwu, 2012). It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. Bacterial infections are prevalent in most parts of Kenya and have resulted to the emergence of antimicrobial-resistant strains of pathogens. This resistance is caused by exposure to microbes carrying resistant genes, spread of resistant microbes in hospitals due to lack of hygiene, exposure to sub-optimal levels of antimicrobial inappropriate antibiotic use because resistance is basically a genetic mechanism that enables the strains that can avoid the mode of action to survive leading to mutation since they may change their genetic code while some resistance may also be related to phenotypic characteristics. There are more than a thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits (Ugwuona, 2014).

These phytochemicals play a number of roles in both plant and animal biological processes. For example, some phytochemicals exhibit

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antioxidant activity, hormonal action, enzymes stimulation, interference with DNA replication, antibacterial activity and many other physical activities (Omojate *et al*., 2014).

This project however intends to focus on the phytochemical and antimicrobial activities of ornamental plants (Mocan *et al.,* 2017); *Iris versicolor* and *Nerium oleander* against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

The word is derived from the Greek word phyto which means plant chemicals produced by plants through primary or secondary metabolism (Mendoza & Silva, 2018). They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens or predators. In the pharmaceutical industry, plants represent the main source to obtain various active ingredients which exhibit pharmacological effects applicable to the treatment of bacterial and fungal infections and also chronic degenerate diseases such as diabetes and cancer (Rodriguez-Garcia *et al.*, 2017). In the history of humanity plants have always been present as a source of health. The knowledge of the various healing properties of plants has been transmitted in an empirical way (Allahverdiyev *et al.,* 2011). However over time man has been interested in knowing where the properties of plants come from hence in the process of knowledge generation man has developed many methodology to know the structures of organic responsible for the healing properties of plants (Saini *et al.,* 2016). This is the birth of phytochemistry which is defined as the science responsible for the study of the compounds contained in plants. They are sometimes called phytoconstituents and protect plant against infections or infestation. The study of phytochemicals and other natural products is called phytochemistry (Yashin *et al.,* 2017). Through phytochemistry, several phytochemicals have been isolated and characterized from various plant species. There are several classes of phytochemicals that include:

Table 1 Classes of Phytochemicals

phytochemicals	Description of the phytochemicals	Citation
Alkaloids	These are the largest group of phytochemicals made largely of ammonia compounds comprising basically of nitrogen bases synthesized from Namino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen. The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue	(Alorkpa,*et al* 2016)
Glycosides	These are the condensation products of sugars with a host of different varieties of organic hydroxy compounds, in such a manner that the hemiacetal entity of the carbohydrate must essentially take part in the condensation. Glycosides are colourless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulfur) water-soluble phytoconstituents, found in the cell sap. Chemically, glycosides contain a carbohydrate (glucose) and none carbohydrate part (aglycone or genin) Alcohol, glycerol or phenol represents aglycones. Glycosides are neutral in reaction and can be readily hydrolysed into its components with ferments or mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of a glycine or pharmacological action.	(Doughari, 2012) (Duan, *et al*, 2019) (Kren & Martínková, 2001).
Flavonoids	These are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure and numerous reports support their use as antioxidants or free radical scavengers. The	(Re *et al.*, 1999)

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	compounds are derived from parent compounds known as flavans.	
Phenolics	These are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL) They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections.	(M Varoni,*et al,* 2012).
Saponins	These possess 'soap like' behaviour in water, i.e. they produce foam. On hydrolysis, an aglycone is produced, which is called sapogenin	(Augustin, *et al,* 2011)
Tannins	These are phenolic compounds of high molecular weight that are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather They are acidic in reaction and the acidic reaction is attributed to the presence of phenolics or carboxylic group.	(Doughari, 2012).
Terpenes	These are the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins (Raut & Karuppayil, 2014)	
Anthraquinones	These are derivatives of phenolic and glyosidic compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols	(Patel & Patel, 2016).
Steroids	These are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal.	Doughari, 2012)
Essential oils	These are the odorous and volatile products of various plant and animal species. Essential oils have a tendency to evaporate on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils. They mostly contribute to the odoriferous constituents or 'essences' of the aromatic plants that are used abundantly in enhancing the aroma of some spices.	(Doughari, 2012)

Study Area

The study was done at Kabianga location in Kericho County, and the collection of the plants, *Nerium Oleander* and *iris versicolor* was carried out within the University of Kabianga and Kapmaso village respectively. The University is situated in the famous and lush tea growing highlands of Kericho in the Southern-Western end of the Rift Valley province of Kenya and within the proximity of the famous multinational tea growing companies, Unilever, James Finlay and George Williamsons. The vegetation of Kabianga is an evergreen land with moderate rainfalls of 2,125 mm p.a being highest and 1400 mm p.a in lower parts while the temperature range is between 10 ºC-29 ºC. The agricultural activities involved mainly are tea farming, dairy and poultry keeping. It is home to the best of Kenyan tea which is renowned worldwide for its taste. Kabianga coordinates are 0.4339 º S, 5.1324 ºE

The experimental design was used in the study discussed there in. Mature *Iris versicolor* and *Nerium oleander* were selected and collected in a simple random sampling technique from

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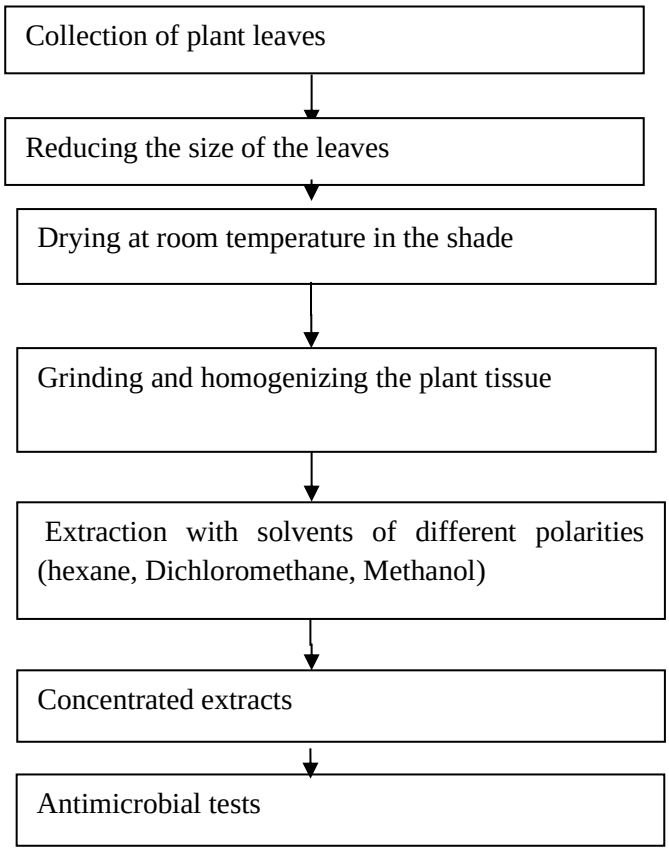
University of Kabianga and Kapmaso village regions where leaves, flowers and roots of the plucked plants were placed in sampling bags and transported to the botany lab of the University of Kabianga laboratory. In the laboratory, the leaves, flowers and roots were dried, and a few samples were pressed.The pressed samples were taken for identification at the Botany Department of the National Museums of Kenya. The leaves, flowers and roots were then dried at room temperature, crushed and milled into fine particles. The fine particles were then extracted separately using hexane, dichloromethane: methanol (1:1) and methanol. Extracts were then exposed to different bacterial strains to evaluate antimicrobial sensitivity testing.

MATERIALS AND METHODS

Solvent Extraction of secondary metabolites from leaves flowers and roots.

The grounded leaves, flowers and roots were separately solvent extracted as shown in Figure 1 below: 500 grams of the powder was weighed separately and put into 2.5L reagent bottle, and then 1.5L of the solvent was added, and the bottle corked. The corked bottles were swirled to ensure that the whole powder was submerged. It was left to stand for 72 hours before filtration. The mixture was then filtered using Whatman filter paper no 1, and the filtrate was collected using a conical flask. The filtrate was then evaporated under vacuum, and dried to a constant weight (W1). The yield of the extract is evaluated as a percentage (%) of the initial weight of the grounded leaf, flower and root powder. The dry extract thereafter stored in a 10ml beaker covered with a parafilm and kept at 4°C awaiting use. The residue was dried for the subsequent extraction using solvents of different polarities. The extracted mass appeared greenish for the leaf extract, and brownish for the flower and root extract. The concentrated gummy semi-solid mass of the extract was then coded and labeled for identification purposes and then stored aseptically in a refrigerator awaiting use. The procedure was repeated for ground flowers and roots. Extraction followed the following schematic diagram.

Figure 1: Schematic diagram (Author 2024)



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Determination of Phytochemicals present in the two ornamental Plants

The two plants were screened for presence of phytochemicals as follows; the plant material was homogenized and treated with solvents of different polarities to extract different bioactive molecules. Later on, the extracts were identified for different classes of compounds present by treating with different reagents for different compounds present.

Phytochemical component

Test for Tannins

To 1ml of the extract, 2ml of 5% ferric chloride was added; forming of dark blue or greenish black which indicate the presence of tannins (Maheshwaran *et al*., 2024).

Test for saponins

2ml of extract and 2ml of distilled water was mixed and shaken in a graduated cylinder for 15 minutes to mix. A formation of a 1cm layer of foam indicated the presence of saponins (Islam *et al*., 2023).

Test for alkaloids

To 2ml extract, 2ml of concentrated sulphuric acid and a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicated the presence of alkaloids (Maheshwaran et al., 2024).

Test for flavonoids

To 2ml of extract, 1ml of 1ml of 2 N sodium hydroxide (NaoH) was added and mixed thoroughly. The presence of yellow color indicated the presence of flavonoids (Kukreti *et al.*, n.d.)

Test for Terpenoids

1ml of ethyl acetate was added to 0.5g of the extract, at that point 2ml of chloroform was added to the mixture and then shaken vigorously.3ml of concentrated sulphuric acid was carefully added alongside under layering the mixture. The presence of a reddish-brown coloration at the interface indicated the presence of terpenoids (Khoee & Madadi, 2023).

Test for Phenols

The extract was screened for phenols by addition of 1ml ferric chloride to 2ml of the extract. The formation of blue to green color indicated the presence of phenols (Sarkar, n.d.)

Test for steroids

0.5g of the extract was dissolved in 2ml chloroform, 3ml concentrated sulphuric acid was carefully added alongside the test tube to form an underlayer. A reddish-brown colour at the interface indicated the presence of a steroidal ring (KUBER, 2023).

Test for gylcosides

In 2 ml of extract, 3 ml of chloroform and 10 % ammonia solution were added and mixed well. The formation of pink color indicates the presence of glycosides (Godlewska *et al*., 2023).

RESULTS

Determination of phytochemicals present in leaves, flowers and roots of Iris versicolor and Nerium oleander

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Table 2 phytochemicals present in plant extracts

Phytochemicals	Hexane				DCM				Methanol			
	N. oleander		*I. versicolor*		*N.oleander*		*I.versicolor*		*N. oleander*		*I.versicolor*	
	Leaves	**Flowers**	**Leaves**	**Roots**	**Leave**	**Flowers**	**Leaves**	**Roots**	**Leave**	**Flowers**	**Leaves**	**Roots**
Alkaloids	+	+	-	+	+	+	-	+	+	+	-	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	-	-	+	-	-	-	+	+	-	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	-	-	+	-	-	-	+	+	-	+
Phenols	-	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	-	-	+	+	-	-	+	+	-	-

The phytochemicals present (+) in the results above has been known for therapeutic activity including microbial activity.

DISCUSSION OF RESULTS

The test results of the three solvents used for the extraction of the phytochemicals from the crude extracts showed that there is gradient elution of the phytochemicals increasing from Hexane, DCM and finally Methanol as per to their polarity features. This is because most phytochemicals were present from the methanol extract as seen in *Nerium Oleander* leaves, flowers and the *Iris versicolor* leaves and roots methanol extracts compared to DCM and Hexane extract of the same plants.

The polarity of the solvent used to extract the compounds determines the kind of bioactive compounds that was extracted and screened. Methanol is a polar solvent used to extract bioactive compounds from extracts when compared to other solvents.

Phytochemical screening not only helps to reveal the constituents of the plant extracts and the one that predominates over the others but also is helpful in searching for bioactive agents that can be used as dietary supplements. Considering the above results, it can be concluded that phytochemicals are biologically active compounds including tannins, saponins, alkaloids, phenols, terpenoids, steroids, flavonoids and gylcosides that possess a wide spectrum of biological activities and thus conduct biological functions that enhance therapeutic activities such as anti-inflammatory ,anti-oxidant, anti-mutagenic and ,anti-carcinogenic properties. Thus the active components could be further taken for investigation and research and hence the outcome of the research could be fruitful in developing potent drugs against various diseases. From the literature and previous research findings, the flavonoids, polyphenolic compounds provide anti-oxidant activity by acting as oxygen-free radical quenching inhibition of lipid peroxidation. Also the other hand flavonoids act as anti-cancer through inhibition of tumor thus inhibiting the development of lung cancer. In addition, terpenoids, alkaloids and phenolics act as antibacterial as well as antifungal by inhibiting micro-organisms thus reducing risk of infections. Also the phenols from previous studies has shown detoxify ability in the body as it increases urination and bile production as well as having mild laxative effect. The roots have been used as inflammatory, diuretic cathartic, diaphoretic and emetic. From the literature as well *iris versicolor* has been used as a laxative and to relieve fluid retention and bloating, as well as treat swelling (inflammation) and skin conditions and preventing vomiting in humans and liver treatment. The native American groups used dried rhizome of the iris plant in small amounts as carthartic and diuretic and as well as powdered iris added to a perfume. In addition, the *N.oleander* has been used for immune boosting action as well as inhibition of angiogenesis in cancer cells and causing apoptosis(natural cell death) in cancerous

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cells. From research findings also, in western sahara, the ash from *N.oleander* is mixed with saltpeter to make gun powder though from historical records, the use and properties of these plants for medicinal use were known by the Babylonians ,Egyptians ,Assyrians and Hebrews while in Kenya in the Eastern province Embu and Mbeere districts the are equipped with a diversity of herbal medicine for treating several diseases by local herbalist while the Samburu made soup from natural herbs, berries and other wild fruits. From other literature findings, the Nandi community used medicinal plants to treat various human and livestock ailments while the aluos used medicinal plants to manage certain cultural problems which may not respond to conventional practices.

CONCLUSION

Methanol is polar in nature due to the unequal charge distribution of the atoms and its asymmetric molecular geometry. Oxygen is more electronegative than Hydrogen and Carbon gain partial negative charge (-) while hydrogen and carbon get partial positive charge (+) hence for this reason methanol has high extractability thus good penetration to the cell content. Methanol has a bend in its shape, thus with the negative end of the net electric dipole moment pointing towards the oxygen atom, thus is capable of extracting both lipophilic and hydrophilic molecules and substances as well it is removed easily at room temperature because it is highly volatile.

Dichloromethane is also polar molecule as individual bond dipoles do not cancel each other, the molecule has tetrahedral geometry hence the shape electronegativity and dipole moment confirm the DCM to be polar, therefore it is able to dissolve a wide range of organic compounds thus these properties combined with its volatility makes it highly effective solvent.

Hexane on the other hand is non-polar solvent with a boiling point of 68^0C and as well unbranched alkane containing six carbon atoms thus become a neurotoxin and volatile organic since the electronegativity difference between the hydrogen and carbon atoms is very small.

RECOMMENDATIONS

The plants leaves, flowers and roots extract can be used in the formulation of a drug against the bacterial micro-organisms after scientific validation of their safety. There is need to elucidate phytochemical components present in the extracts which might be responsible for the antimicrobial activity.

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