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Research article

PHYTOCHEMICAL SCREENING OF *AGAVE SISALANA* PERRINE LEAVES (WASTE)

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ABSTRACT: Phytochemical properties of the methanolic, Ethyl acetate and Hexane extract of the *Agave sisalana* Perrine leaves were investigated to evaluate the chemical properties. The phytochemical screening revealed that Tannins, Cardiac glycosides, Reducing sugars, Saponins, Flavonoids, Phlobatannins, Steroids, Terpenoids, and Coumarins were present in the three extracts of A. *sisalana* Perrine leaves while, Alkaloids were present only in the methanolic and Ethyl acetate extracts. Anthraquinones and Emodins were present only in methanolic extract, while Anthocyanins were absent in all the three extracts. The study revealed that *A. sisalana* Perrine leave juice (waste) has potential Phytochemical compounds which could be investigated for antimicrobial activities for treatment of pathogenic organisms.

Key words: *Agave sisalana*, Phytochemical, secondary metabolite, decortications, fibre

INTRODUCTION

Agave sisalana Perrine, popularly known as sisal, belonging to the Agavaceae family and is monocotyledonous (Santos et al., 2009). *Agave sisalana* Perrine, occupies the 6th place among fibre plants, representing 2% of the world's production of plant fibres (plant fibres provide 65% of the world's fibres). Agaves can be grown on arid and semi-arid lands not suitable for other lignocellulosic feedstocks, such as switch grass and sugarcane. In addition, although agave species are native to the American continent, they have worldwide potential for production (Davis et al., 2011; Garcia-Moya et al., 2011). According to Bisanda et al., (2003) Sisal was introduced in Tanzania in 1893, from where it spread to other parts of East Africa and fibre production had started by 1898. Since the introduction of sisal, the production method of sisal fibres has remained the same, where the industry has only been harvesting leaves from which the hard fibre is extracted. The sisal fibre is obtained from the leaves of the plant by wet or dry decortications, using machines, a process which involves crushing the leaves between rollers, scraping the resulting pulp from the fibre, washing and then traditionally drying in the sun. In a Tanzania sisal processing factory according to Raymond et at., (2013) as well as Mshandete et al., (2008), only 2 % of the sisal plant is used as fibre while the remaining biomass after decortications is dumped near the factories an observation reported earlier by Mashauri *et al*., (2004), this practice cause serious environmental problems. Recent studies have indicated utilization of Sisal leave decortications residues for biogas production (Muthangya et al 2009); Cultivation of Oyster Mushroom (*Pleurotus* HK-37) on Solid Sisal Waste (Raymond et al., 2013) and biogas production from sisal pulp waste (Mshandete et al., 2005). Sisal waste has also been used as fertilizer (Lacerda et al., 2006), pesticides (Baker, 2003) and also animal feed (Faria et al., 2008). Pizarro et al. (1999) reported that the sisal waste has insecticidal properties particularly against larvae of mosquitoes.

The search for natural products from agro-industrial waste, which may became useful to society, has grown in recent years. Plants are known to be rich in secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found to have antimicrobial properties *in vitro*. This study was designed to investigate the presence of secondary metabolites in *Agave sisalana* Perrine leaves waste which were successively and exhaustively extracted using three different solvents.

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MATERIALS AND METHODS

Sisal leaves of agave hybrid 11648 were collected in sterile autoclavable plastic bags from the Kilifi Plantation Limited, a Sisal Estates located at the Northern part of the Kenyan Coastal line. Leaves were washed using Sterile Distilled Water (SDW), dried under shade on a Sterile Blotter for ten (10) days and chopped into 1.5-2cm cubes, the cubes were blended into fine powder in the laboratory.

Phytochemical screening

Extraction of extracts

The powder stored in labelled air tight polythene bags at 4˚C away from light. 250g of the powder was successively and exhaustively extracted by maceration using one liter each of hexane (C_6 H_{14}), Ethyl acetate (EtOAc) and methanol (CH_3OH) solvents. Other study by Flavia et al. (2008), reported more compounds from methanol extract when compared to aqueous extraction, an observation which lead to elimination of aqueous extraction in this study and inclusion of hexane and ethyl acetate. Filtration was done using Whatman No.1 filter paper. The solvent extracts were concentrated under reduced pressure using a rotary evaporator and preserved at 4˚C in air tight bottles according to Savithramma et al., (2011) with modifications.

Preliminary qualitative phytochemical screening

Preliminary qualitative phytochemical tests were carried out on the extracts using standard procedures to identify the presence of tannins, cardiac glycosides, reducing sugars, saponins, flavonoids, phlobatannins, steroids, terpenoids, coumarins, alkaloids, anthroquinones, emodins and anthocyanins as detailed below.

Test for Alkaloids

Alkaloids were tested according to Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011) with modification. About 100 mg of each extract was warmed with 2mL 2% H_2SO_4, for two minutes. It was then be filtered and a few drops of Dragendorff reagent added. An orange red precipitate indicated presence of alkaloids. Dragendorff reagent A consists of 170 mg basic bismuth nitrate in 100 mL solution of water/acetic acid in the ratio of 4:1, while Dragendorff's solution B consists of 40 g of KI in 100mL of water. The Dragendorff reagent will consist of solutions mixed in the ratios of 5 mL solution A: 5mL solution B: 20 mL acetic acid: 70 mL water.

Test for Tannins

Tannins were tested according to Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011) with modification. About 100 mg of each extract was mixed with 2 mL of distilled water and heated on water bath then filtered. About 2 drops of 1% Ferric Chloride solution in methanol (1:1) was added to the filtrate. A dark green solution indicated the presence of tannins.

Test for Anthraquinones

About 200 mg of each extract was boiled with 3mL of 10% HCI for three minutes in a water bath. It was then filtered and allowed to cool. Equal volume of $CHCI_3$ was added to the filtrate. Few drops of 10% ammonia were added to the mixtures and heated. Formation of a rose prink colour indicated the presence of anthraquinones, a procedure adopted from Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011).

Test for Cardiac Glycosides (Keller-Killani Test)

Cardiac glcosides were tested according to Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011) with modification. About 200mg of each extract was shaken with 5mL distilled water and filtered. About 3mL of extract was treated with 2mL glacial acetic acid containing one drop of 1% ferric chloride solution. This was underlayed with 1mL of concentrated H_2SO_4 acid. A brown ring of the inter-phase accompanied by a violet ring below it indicated presence of a deoxysugar, a characteristic of cardenolides

Test for Reducing Sugars

The reducing sugars were tested according to Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011) with modification. About 100mg of each extract was shaken with 3mL distilled water and filtered. The filtrate was then boiled with 2 drops each of Fehling solution A and B for five minutes. An orange red precipitate indicated presence of reducing sugars. Fehling solution A will consist of $CuSO_4.5H_2O$ and H_2O in the ratio of 7:93, while Fehling solution B consists of Potassium sodium tartarate 20%, H_2O 65% and NaOH 15%.

Test for Saponins

About 100mg of each extract was shaken with 3mL of distilled water and heated to boil. Frothing or appearance of creamy miss of small bubbles showed the presence of saponins as described by Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011).

Test for Flavonoids

About 100mg of each extract was added to 1mL of 0.1M NaOH, then 1mL of 0.1M HCI added. A yellow solution that turned colourless indicated presence of flavonoids as described by Mohammad *et al.*, (2011) and Savithramma *et al.*, (2011).

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Test for Phlobatanins

About 100 mg of each extract was shaken with 3mL of distilled water and then filtered. The filtrate was then be boiled with 1mL 2% HCI solution. Red precipitate showed the presence of phlobatanins as described by Mohammad *et al.*, (2011) and Savithramma *et al*., (2011).

Test for Steroids

About 100mg of each extract was shaken with 3 mL of $CHCl_3$ followed by 2 mL concentrated H_2SO_4 added by the sides of the test tube. When the upper layer turned red and the H_2SO_4 layer showed a green fluorescence, it indicated presence of steroids as described by Mohammad *et al.*, (2011) and Savithramma *et al*., (2011).

Test for Terpenoids (Salkowski Test)

About 100mg of each extract was mixed with 1mL of chloroform ($CHCI_3$) followed by 2mL of concentrated H_2SO_4 carefully added by the sides of the test tube to form a layer. A red-brown colouration of the interface indicated positive results for the presence of terpenoids as described by Mohammad *et al.*, (2011) and Savithramma *et al*., (2011).

Test for Anthocyanins

About 100mg of each extract was shaken with 3mL of distilled water and filtered. To 1mL of the filtrate, 1mL each of 2M HCl and 2M NH_4OH were added. The appearance of pink-red colour that turned blue-violet indicated the presence of anthocyanins as described by Savithramma *et al*., (2011).

Test for Coumarins

About 100mg of each extract was shaken with 3mL of distilled water and filtered. To 1mL of the filtrate, 1mL of 10% NaOH was added. Formation of a yellow colour indicated presence of coumarins as described by Savithramma *et al*., (2011).

Test for Emodins

About 100mg of each extract was shaken with 3mL of distilled water and filtered. To 1mL of the filtrate, 1mL NH_4OH and 2mL of Benzene were added. Appearance of red colour indicated presence of emodins as described by Savithramma *et al*., (2011).

RESULTS AND DISCUSSION

The preliminary Phytochemical screening results of *Agave sisalana* Perrine leaves juice showed the presence of various bioactive secondary metabolites constituents (Table 1). The screening showed that, the leave juice contained: Alkaloids, Tannins, Anthraquinones, Cardiac glycosides, Reducing sugars, Saponins, Flavonoids, Phlobatanins, Steroids, Terpenoids, Anthocyanins, Coumarins, and Emodins. These results have some similarity to that reported by Ade-Ajayi *et al*., (2011) working on *Agave sisalana* Perrine juice (waste) collected directly after sisal decortications and extracted using methanol. These components have been reported by Hentschel (1995) to posses' curative activity against several human problems such as diuretic, choleretic, spasmodic, chronic eczema, diarrhea, dysentery and menstrual disorders.

Table 1: Phytochemical screening of the different parts *Agave sisalana* Perrine leaves extract

Plant compound	Extract		
	Methanol	Ethyl acetate	Hexane
Alkaloids	+	+	-
Tannins	+	+	+
Anthraquinones	+	-	-
Cardiac Glycosides	+	+	+
Reducing sugars	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Phlobatanins	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Anthocyanins	-	-	-
Coumarins	+	+	+
Emodins	+	-	-

The available secondary metabolites in the *Agave sisalana* Perrine leaves extract shows that, the extract may have some medicinal potential. This can be attributed to the fact that most of the components identified have been documented to posses' therapeutic usage. For instance, plants rich in saponins are known to be immune boosting and have anti-inflammatory properties as reported by Kenner and Requena, (1996). Saponins from different sources have been found to be detrimental to Protozoa. (Newbold et. al., 1997). Sen et. al., (1997) reported that saponin influence both ruminal bacterial species and number through specific inhibition or selective enhancement of growth of individual species. Similarly, plants with tannins have antibacterial potentials (Elmarie and Johan, 2001), while activities of alkaloids and flavonoids have been reported by Onwuliri and Wonany (2005). According to Spargs (2004), chemical classes of anti-inflammatory agents from natural sources have been usually reported to engage a vast range of compounds such as polyphenols, flavonoids, terpenoids, alkaloids, anthraquinones, lignins, polysaccharides, saponins and peptides. This study clearly indicates that, methanol extract contained more metabolites compared to the ethyl acetate as well as hexane extracts. Similar observation on methanol extraction have been reported by Flavia et al. (2008) when comparing methanol and aqueous extraction, an observation which lead to elimination of aqueous extraction in this study and inclusion of hexane and ethyl acetate.

Therapeutic agents suitable for the treatment of chronic inflammatory diseases are highly desirable in the developing countries, which has resulted in an increased interest in complementary and alternative medicines due to the increasing resistance of micro organisms to conventional medicine. Application of herbal species traditionally as medicines against inflammatory ailments have been practiced world over, with some of the herb studied and reported to posses the properties (Ade-Ajayi et. al., 2011). The results of this study indicate possible curative properties from the extracts. These results suggest that the bioactive compound(s) of the extract studied here could be exploited for commercial application. At present, this residue is discarded by the sisal farms, but could constitute a potentially useful raw material, which when stabilized and treated adequately, could be useful in the treatment of diseases as well as reduction of environmental pollution caused by the waste.

CONCLUSION

The results of this study reveal the presence of some of the phytochemical components in *A. Sisalana* leave juice which could have a potential of being human immune boosters as well as anti inflammatory agents. Quantitative analysis of the phytochemical components needs to be done as well as antimicrobial screening for the potential application in medicine for treatment against pathogenic organisms.

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