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Phytochemical Screening and Antimicrobial Effects of Aloe Vera Juice on Some Common Skin Pathogens

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Abstract

Phytochemical and antimicrobial screening of Aloe Vera Juice was analyzed, after plant sample was extracted using methanol and distilled water. The result of qualitative phytochemical screening of the aqueous extract revealed the presence of alkaloids, steroids, saponins, phenols, flavonoids, reducing sugars, tannins and terpenoids. The antimicrobial potential of the extracts was tested against Staphylococcus aureus, Streptococcus pyogenes and Candida albicans, the extracts were prepared at different concentrations of l00mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml. The antimicrobial activity was determined after incubation period by measurement of mean diameter zones of inhibition produced by the extracts against the test organisms. The result showed high antimicrobial activity against Staphylococcus aureus and Streptococcus pyogenes and low inhibition activity on Candida albicans while no antibacterial activity on methanol extracts.

Key Words: Phytochemical, Antimicrobial, Aloe Vera Juice

Introduction

Over the years plant and their extracts have been applied as herbal remedy for diverse human ailments. Presently, plants are still being utilized by numerous developing countries as sources of therapeutic agents because they believe medicinal plants are readily available, accessible, affordable, with potentially relatively lower incidence of adverse reaction compared to modern conventional drugs (Ogu et al., 2012). The use of medicinal plant as a source for relief from illness can be traced back over five millennia to written document of the early civilization in China, India, and the near east, but it is doubtless an art as old as mankind. Neanderthals who lived 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethno medicine around the world. The potentials of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species only a small percentage has been investigated phytochemically, and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Medicinal plants represent rich sources of antimicrobial agents; plants are used medicinally in different countries and are source of many potent and powerful drugs. A wide range of medicinal plant parts extracts are used as raw drug and they possess varied medicinal properties. The different parts used include root, stem, flower, fruits, twigs and modified plant organs. While some of these raw drugs are collected in small quantities by the local communities and folk healer for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated. Plants have the ability to synthesize a

wide variety of chemical compound that are used to perform important biological functions, and defend against attack from predators such as insects, fungi and herbivorous mammals. Many of the phytochemicals have beneficial effect on long term health when consumed by human and can be used to effectively treat human diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive property. They are non-essential nutrients, meaning that they are not required by human for sustaining life, it is well known that plants produce these chemicals to protect themselves, but recent research demonstrates that they can also protect humans against diseases. There are more than a thousand known phytochemicals (Srivastava, 2000). Aloe Vera is rich in phytochemicals which occur naturally in plants and have beneficial effects on health. The gel in Aloe Vera leaves is used typically to help heal wound and minor skin irritations such as frostbites, burns and sunburns as well as skin conditions such as psoriasis, Aloe Vera juice is taken for the treatment of variety of conditions including diabetes, gastro-duodenal ulcer, colitis, enteritis, and immune system deficiencies. Examples of phytochemicals, some of which are found in Aloe vera extract are glycosides, carbohydrates, saponins, tannins phlobatannins, steroids, flavonoids, salicylic acids, phenolics, mannose, aloin and cinnamic acids. The antimicrobial effect of phytochemicals is an established fact and may as well be useful in the treatment of skin infections caused by common skin pathogens. The many layers and structures of the skin serve as a laborite hosts to microbes, including a diversity of commensal and pathogenic bacteria that contribute to both human health and disease. The skin layer is critical for survival, preventing the escape of moisture and invasion by infections or toxic substances (Segre, 2006). The skin is also an intricate habitat for a diverse population of microbes. During the birching process and subsequent exposure to the post-natal environment the skin is colonized by a wide array of microbes, many of which are commensal or symbiotic. Proposed beneficial roles of resident microbiota include inhibition of pathogenic species and further processing of skin proteins, free fatty acid, and sebum. The skin is composed of a variety of niches, including regions with broad range of pH, temperature, moisture, and sebum content. Furthermore, skin structures such as hair, follicles, sebaceous, occrine, and apocrine glands comprise sub habitats that may be associated with their own unique microbiota. Many lines of evidence suggest a role for microorganisms even in non-infectious skin diseases such as atopic dermatitis (AD; eczema), rosacea psoriasis and acne (Till et al., 2000; Paulino et al, 2006). Resident microbial may become pathogenic sometime in response to an impaired skin barrier. The species has a number of synonyms such as Aloe barbadensis mill, Aloe indica Royle, Aloe perfoiiata and Aloe vulgaristan. The species epi'!».et Vcra means true or genuine. Some literature identifies the white spotted form of aloe vera as aloe vera var chinenisis (Wang et al, 2004) Techniques based on some comparison suggest aloe vera is relatively closely related to Aloe perryi, a species endemic to Yemen (Darokar et al., 2003). Similar techniques using chloroplast DNA sequence comparison and 133R profiling have also suggested it is closely related to, Aloe, forbesii, Aloe inermis, Aloe Scobinifolian, Aloe sinkatans and Aloe striata (Treutlen et al., 2003). With the exception of the South African species Aloe striata, these Aloe species are native to Socotra (Yemen), Somalia and Sudan. The lack of obvious natural population of the species has led some authors to suggest Aloe Vera may be of hybrid origin (Jonas et al., 2002)

Aloe Vera Distribution

The natural range of aloe vera is unclear as the species has been widely cultivated throughout the world. Naturalized stand of the species occurs in the southern, half of the Arabian, peninsula through North Africa (Morocco, Mauritania, Egypt) as well as Sudan and neighboring countries, along with the Canary Capa Varde and Madeira Islands. This distribution is somewhat similar to the one euphorbia balsamifera pistacia atlentica, a few other suggesting that a dry sclerophyll forest once covered large areas but has been grammatically reduced due to desertification in the Sahara leaving these few patches isolated several closely related (or sometime identical) species can be found on the two extreme sides of the Sahara dragon trees Dracoena and aeomium being two of the most representative examples. The species were introduced to China and various plants of southern Europe in the 17th century. The species is widely naturalized elsewhere occurring in temperate and tropical regions of Australia Barbades Belize, Nigeria, Paraguay and United States (Akinyola and Odiyi, 2007). The actual species distribution has been suggested to be the result of human cultivation (Akinyele and Odiyi, 2007).

Cultivation of Aloe Vera

Aloe Vera has been widely grown as an ornamental plant, the species is popular with modern gardens as a putatively medicinal plant and for it's interesting flowers form and succulence. Thus, succulence

enables the species to survive in areas of low natural rainfall, making it ideal for rockeries and other low water-use gardens (Yates, 2002). The species is relatively resistant to most insect pest in pots; the species requires well-drained sandy potting soil and bright sunny condition. Terra cotta pots are preferable as they are porous (Coleby-Williams, 2008), potted plants are allowed to completely dry prior to re-watering when potted, aloes become crowded with pups growing from the sides of the mother plant, they are usually divided and repotted to allow room for further growth and help prevent pest infestation during winter. Aloe Vera may become dormant during which little moisture is required in areas that receive frost or snow. Production of aloe vera is undertaken in Australia, Bangladesh, Cuba, the Dominican Republic, China, Mexico, India, Jamaica, Kenya, Tanzania and South Africa along with USA to supply the cosmetics industry with aloe vera gel. This plant has gained the royal horticultural society's award of garden merit.

Chemical Composition of Aloe Vera Plant

The aloe plant being a cactus plant is between 88 and 99.5% water with an average PH of 4.5. The remaining solid material contains over 75 different ingredients, some of the most desirable constituents because of their well-known health supporting benefits are the polysaccharides or glucomannans, the glycoproteins arc associated with growth factors (Behl et al., 1993). The aqueous extracts of aloe vera have been found to contain anthraglycosides, reducing sugars and cardiotonic glycosides. In the ethanol extraction, saponins, carbohydrates sterols, triterpenoids and anthroquinone have been found (Vasquez et al., 1996). It was reported that aloe vera contains over 39 essential mineral and vitamins and all the 23 amino acids, the different ingredients including vitamins, minerals, enzymes, sugars, anthroquinone phenolic compounds, liqnins, saponine sterols, amino acid and salicylic acid are thus described in more detail below:

Vitamins:

The plant contains many vitamins, they include the important antioxidant vitamin A, C and E, vitamin B, (Thiaminc) Niacin, vitamin 82 (Riboflavin) chorine, folic acid and vitamin Be, some authorities suggest that there is also a trace of vitamin Bn (Hardman, 2001).

Enzymes:

When taken orally several of these biochemical catalysts such as amylase and lipase can aid digestion by breaking down fat and sugars, one important enzyme carboxyl peptidase inactivated bradykinin and produces and anti-inflammatory effect. During the inflammatory process bradykinin produce pain, associated with vase-dilatation and therefore, its hydrolysis reduces these two components and produce arc analgesic effect. Other enzymes include SGO transaminase SGP transaminase and lactic dehydrogenate.

Minerals:

Sodium, potassium, calcium, magnesium, manganese, copper, zinc, chromium, selenium and iron are all found in the aloe vera plant (Allen and Pilbeam, 2007). Magnesium lactate inhibits histamine decarboxylase and prevents the formation of histamine from the amino acid histamine. Histamine is released in many allergic reactions and causes intense itching and pain. The prevention of its formation may explain the antipuritic effect of aloe vera.

Sugars:

Sugars are derived from the mucilage layer of the plant under the rind, surrounding the inner parenchyma or gel. They form 25% of the solid fraction and comprise both mono and polysaccharides. By far the most important are the long chain polysaccharides comprising glucose and mannose known as glucomannans (Beta -1, 4- linked acetylated mannan) (John et al, 2009). When taken orally some of these binds to receptor sites lining the gut and form a barrier possibly helping to prevent leaky gut syndrome, other are ingested whole by a method of cellular absorption. The polysaccharide is absorbed complete and appear in the blood stream unchanged. There, they act as iiTHrmne-modulative capable of enhancing and retarding the immune response (Lee et al., 2004). Acetomannan is the major carbohydrate fraction obtainable from the gel of aloe vera. This has been analyzed so as to determine the quality of the plant. A glycopeptides named GIGLLMIDIZ has been isolated from the gel of aloe vera. The carbohydrate and protein contents of the glycopeptides were 20.9% and 32.6% respectively (Yang et al, 1998). The carbohydrate variety composed of fructose, galactose, glucose and mannose.

Materials and Methods

Materials

Aloe vera leaves, distilled water, sterile blade, beakers, filter paper, refrigerator, stirrer, nutrient agar, potato dextrose agar, Muller Hinton agar, Petri dishes, transparent meter rule, pipettes, conical flasks, reagent bottles, measuring cylinder, test tubes

and test tubes rack, heating mantle, weighing balance, spatula, funnels, sterile swap, masking tape, autoclave, incubator, stove, round bottom flask, water bath, wire loop test tube holder, sample bottles, volumetric flasks, vacuum pump.

Reagents and Chemicals

Hydrochloric acid (HC1), Mayer's and Wagnes reagents. 10% ferric chloride (FeCL3), Chloroform, methanol, sulphuric acid (H2SO4), lead acetate solution, Fehling's solution A and B, Iodine, Benzene, 10% ammonia, aqueous (NCsC3) aq and acetic acid.

Procedures

The work was mapped out in four steps namely;

- Plant sample collection and identification
- Extraction of sample
- Phytochemical screening of plant extracts
- Determination of antimicrobial activity of extracts

Collection and Identification of Plant Sample

The plant sample was collected from a privately-owned garden in Samaru. The identity of the plant was authenticated in Herbarium Department of Biological Sciences, Ahmadu Bello University Zaria.

Preparation of Plant Sample

The plant sample was rinsed in clean water. A sterile blade was used to cut the leaves into small pieces and blended into fine gel. The gel was then stored in a beaker (Hernandez et al, 1999)

Aqueous Extraction of the Gel

Exactly 250ml of the gel was mixed with 250ml of distilled water and allowed to stay for 24 hours with occasional stirring, it was then filtered. The filtrate was evaporated to dryness and stored in a refrigerator at 4°C (Hernandez et al, 1999).

Methanol Extraction of the Gel

250ml of the gel was mixed with 250mi of methanol and allowed to stay for 24 hours with occasional stirring, the mixture was then filtered and the filrrate was evaporated to dryness and stored at 4°C (Hernandez et al, 1999).

Phytochemical Screening of Extract

The aqueous extract of aloe vera was tested for the presence of phytochemicals such as alkaloids, tannins, steroids, flavonoids, saponins, terpenoids glycosides, carbohydrates, phenols, reducing sugar (Sofowora, 2012; Harborne, 1973 and Ogbuewu, 2008).

Test for Alkaloids:

To 3ml of extract, 3ml of HC1 was added and stirred in a water bath. Mayer's and Wagner's reagent were also added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for Tannins:

1ml of extract was diluted with 4ml of distilled water and few drops of 10% ferric chloride solution added, a blue (or green) colour indicated the presence of tannins.

Test for Steroids:

2ml of the extract was dissolved in 2ml of chloroform and 2ml of concentrated sulphuric acid added in test tube. A red colour produced in the lower larger level indicated the presence of steroids.

Test for Flavonoids:

To 1ml of extract, 1ml of lead acetate solution was added. The formation of yellow precipitate was taken as a positive test for flavonoids (Ogbuewu, 2008).

Test for Saponins:

5ml of extract was mixed with 5ml of distilled water and shaken vigorously; the appearance of a persistent froth (foam) that lasted for 15 minutes indicated the presence of saponins (Sofowora, 2012).

Test for Glycosides:

10ml of 50% H2SO4 was added to 10ml of the extract in a test tube, the mixture was heated in boiling water for 30 minutes. 10ml of Fehling's solution was also added and the mixture boiled. A brick red precipitate observed indicated the presence of glycoside.

Test for Terpenoids:

5ml of extract was mixed with 2ml of chloroform and 3ml of concentration H2SO4 was added. A reddish-brown precipitate at the interface indicated the presence of terpenoids.

Test for Carbohydrates: . -.

To 3ml of extract, I ml of iodine solution was added; a purple coloration at the interphasc indicated the presence of carbohydrates.

Test for Phenols:

2ml of extract was added to 2ml of ferric chloride solution. A deep blue green solution indicated the presence of phenol. (Sofowora, 2012)

Test for Reducing Sugar:

20ml of extracts was added to 5ml of mixture of equal volume of Fehling' solutions A and B and boiled on a water bath for 2 minutes, a brick red colour at the bottom of the test tube indicated the presence of reducing sugar

Antimicrobial Activity of Extracts

Test Organisms:

The test organisms used for this analysis were clinical isolates of bacteria and a fungus obtained from department of microbiology, Ahmadu Bello University, Zaria. The isolates were Streptococcus pyogenes-, Streptococcus aureus and Candida albicans.

Culture Media:

The culture media used for the analysis includes Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), nutrient agar and potato dextrose agar (PDA). The mentioned media were used for sensitivity test, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All media were prepared according to manufacturer's, constructions and sterilized by autoclaving at 121 C for 15 minutes (Rabe and Van 1997).

Determination of Inhibitory Activity (Sensitivity Test) of the Extract Using Agar Well Diffusion Method:

The standardized inoculums of both the bacterial and fungal isolates were streaked on sterilized Mueller hinton and potato dextrose agar plates respectively with the aid of a sterile swab sticks. Four wells were pouched on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentration of the extract prepared which were 100, 50, 25 and 12.5mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract. The inoculated plates with the extract were allowed to stay on the bench for about one hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37°c for 24 hours (plate of Mueller hinton agar) while the plates of potato dextrose agar were incubated at room temperature for about 3-5 days. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured using a transparent ruler calibrated in millimeter and the result was recorded (Ogu et al., 2012 and Elott, 2004).

Determination of Minimum Inhibitory Concentration (Mic).

The minimum inhibitory concentration of the extract was determined using tube dilution method with the Mueller Hinton broth used as diluents the lowest concentration of the extract showing inhibition for each organism when the extract was tested during sensitivity test was serially diluted in the test tubes containing Mueller Hinton broth. The organisms were inoculated into each tube containing the broth and the extract. The inoculated tubes were then incubated at 37°C for 24 hours. At the end of the incubation period, the tubes were observed for the presence or absence of growth using turbidity as a criterion, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC). The result was also recorded.

Determination of Minimum Bactericidal Concentration (Mbc):

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extracts. A sterilized wire loop was dipped into the test tube(s) that did not show turbidity (clear) in the MIC test and a loopful was taken and streaked on a sterile nutrient agar plate. The plates were incubated at 37°C for 18-24 hours. At the end of incubation period, the plates were examined (observed) for the presence or absence of growth. This is to determine whether the antimicrobial effect of the extract is bacteriostatic or bactericidal (Jennette 1985).

Result

Phytochemicals

The results of the phytochemical analysis of Aloe Vera juice presented in table 1 below showed that only phlobatannms is absent in the extracts while alkaloids, tannins, steroid, flavonoids, saponins, carbohydrate, terpenoids, phenol, glycoside and reducing sugar were present.

Table 1: Qualitative analysis of the phytochemicals in Aloe Vera juice

PHYTOCHEMICAL	STATUS (AQUEOUS EXTRACT)
Alkaloids	+
Tannins	+
Steroids	+
Flavonoids	+
Saponins	+
Glycosides	+
Phlobatannins	+
Terpenoids	+
Carbohydrates	+
Phenols	+
Reducing Sugar	+
Keys: + (present) - (absent)	

Antimicrobial Activity Profile

The results of antimicrobial activity of Aloe vera extracts indicated on table 2 shows that aqueous extract shows higher activity, while methanolic extract shows no inhibition which may be caused by wrong handling of sample. Ciprofloxacin and Ketoconazole which served as positive control, Ciprofloxacin with 40mm zones of inhibition for Staphyhcoccus aurcus and Streptococcus pyogenes and also ketacanozolc with 25mm zones of inhibition for Candida albicans, distilled water which served as a negative control showed no zone of inhibition.

Table 2: Determination of Inhibitory activity (Susceptibility test) of Aqueous and Methanolic extracts on the test organisms

	Zone of inhibition at varying Concentration								
Test organisms	**Aqueous extract (mg/mL)**				**Methanolic extract (mg/mL)**		**Controls**		
							Cipro		
	100	50	25	12.5	100	50	25	12.5	l0µg Ketoco500mg Dist.H_{20}
S. aureus	35	30	27	22	0	0	0	0	40 0 0
S. pyogenes	32	30	27	23	0	0	0	0	40 0 0
C. albicans	26	21	18	15	0	0	0	0	25 0 0

Key:
mm = Millimeter
mg = milligram
ml = milliliter
Mg= microgram

Discussion

Medicinal plants play a central role not only in traditional medicine but also as commercial commodities. Literature indicates that medicinal plants are the backbone of the traditional medicine and the antimicrobial activities of the plant extracts are due to different agents in the extracts with antimicrobial compounds (Ogu et al., 2012). In this study the plant extract of Aloe vera was found to inhibit the growth of all the test bacteria and fungi indicating that the plant Aloe vera possesses antimicrobial properties. Aloe vera active ingredients are substances that prevent the growth of disease-causing microorganism and act as a team to provide antimicrobial activity, eliminating many internal and external infections. It is active against bacteria and also helps to treat fungal and viral infections (Boudrcau and Belland, 2006). The inhibition of Candida albicans by this plant extract suggest that it possesses antifungal, properties and thus can be used as antifungal agent for the treatment of refractory Candidiasis. Aloe vera is a major medicinal plant when it comes to treating and protecting the skins, it is very effective on burns and, sunburns, as well as variety of skin diseases like eczema pruritus, psoriasis acne. It is extremely constructive and protective, it speeds- up the process of skin repair, protects from the side effects of radiation and help to speed up the closing of open wounds (Sofowora,2012). Cosmetic companies have profited well by creating numerous creams formulated with Aloe vera used externally. It is also powerful human shield; it protects from infections, reinforces the metabolism and effectively stimulates the immune system. It can also be a good natural means of fighting constipation and all digestive problems (bloating, gas, diarrhea and heartburn). Aloe vera is also good protector of the liver, promotes good blood circulation, combats cholesterol and diabetes, and improves cellular oxygenation. This result could be responsible for popular use of Aloe vera in cosmetic industries. The phytochemical screening result shows that the Aloe vera juice contains alkaloids, tannins flavonoids, carbohydrates glycoside saponins and reducing sugar, phenol and steroid terpenoids while phlobatannins is absent. These compounds may be responsible for their medicinal uses (Sharma and Sharma, 2001). Aloe vera has one of the most amusing compositions consisting like a cactus of more than 99% water, The remaining 1% is very powerful synergy of 150 different elements including vitamins A, B, B2, B3, B12, C and E as well as a large number of minerals and trace elements; Calcium, Sodium, Chlorine, Mangnanese, Copper, Chrome, Zinc, Selenium, Germanium, Phosphorus, Potassium, Iron. Tannins and more than 18 amino acids, the result above may be responsible for the popular use of Aloe vera in cosmetic industries (Sofowora, 2012). The result showed that the aqueous extracts of aloe vera juice have inhibitory effect on Streptococcus pyogenes, Staphylococcus aureus and Candida albicans with zones of inhibition as shown in Table 2,3 and 4 above. They are pathogenic skin disease causing bacteria, this result could be responsible for the popular use of Aloe vera to relief infection caused by these organisms. Aloe vera extracts showed greater inhibitory effect on Staphylococcus aureus, and Streptococcus pyogenes. This result could also be the reason for the use of Aloe vera extract to relief many types of gastrointestinal irritation (Foster, 2010; Grindlay and Reynolds, 2009). The gel is also said to promote wound healing due to the presence of some component like anthroquinone and hormones which possesses antibacterial, antifungal and antiviral activities. The presence of these compounds was confirmed in this study.

References

1) Akinyola, B.O. and Odiyi, A.C. (2007). Comparative study of the vege-morphology and the existing taxonomic status of Aloe Veal L. Journal of Plant Science 2(5).558 -563. DOI; No 3923/jp5.2007.558.563.Allen V.B. and Pilbeam (2007). Handbook of plants nutrition. CRC press. Pp.399.ISBN978-0-8247-5904-9.
2) Behl P.N., Arora R.B., Srivastra G. and Mahorta S.C. (1993). Herbs useful in dermatological therapy (3rd edition). CBS publisher New Delhi, pp.22-24
3) Boudreau, M.O. and Beland F.A (2006). An evaluation obadef the Biological and Taxonomical properties of Aloe barbadensis

(Miller) Aloe vera. Journal of environmental science and health part. (24:103-154).
4) Coleby-Williams J. Act sheet; gardening Australia, Australian Broadcasting Corporationachived. From the original on 6 July 2008, retrieved 008-07-08.
5) Dakora M.P., Rai R., Gupta, A.K., Rajkumar, S., Suneroasan, V., Khanuja, S.P.S., (2003). Molecular assessment of germ plash diversity Aloe spp. Using RADP and AFLP analysis. J Med. Arom. Plant Science (2);354-361.
6) Ellot J.N. (2004). Quantifying the bioactivity plant extracts during screening and bio-assay fractional phytochemical 11:370-371.
7) Foster S. (2010). Aloe vea; the succulent with skin cell protecting properties. Herbs for health medicine, world online wti.//ww.health.net/litray/articles/hfh/aloe.html.
8) Grindlay and Reynolds, (2009). The Aloe vera phenomenon: A review of properties and modern uses gel. Journal of Ethnopharmacol 16-17.
9) Harborne J.B. (1973). Phytochemical methods, a guide to modern technique of plant analysis 4th ed. Pp. 1-5, 182-191.
10) Hardman, J.G. (2001). Goodman and Gilman pharmacology therapeutics, 10th .ed. p.992. ISBN 0071354697.
11) Hernandez, M.M. et al., (1999). Biological activities of crude extracts of Vitis trifolia L. (Verbenacea) Journal of Ethanopharmacology, 67:37-44.
12) John G.V., Catherine G., Barbara N., Elizabeth D., Elizabeth R. (2009). The new oxford book of food plants. Oxford Univesity press US. Pp.212 ISBN 978-0-19-954946-7.
13) Jennette E.H. (1985). Manual of clinical microbiology, 8th Edition. American Association for Microbiology, Washington D.C 178-987.
14) Jonas, W.D. and Sacamano, C. (2002). Landscape plant for dry regions more than 600 species from around the world. California Bills Automotive publishers, USA.
15) Lee S. G., Li L., Earl S.F. and Simin Liu (2004). Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States; an ecological assessment. American Journal of Clinical Nutrition. 79(5):774-779.
16) Ogu, G.I., Tanimowo, W.O., Nwachukwu, P.U. and Igere , B.E. (2012). Antimicrobial and Phytochemical of leaf, stem, bark, and root extract of cycathalaprotratebleme against some human pathogens. Ethanopharmacol, (1) Pp. 35-43.
17) Ogbuewu, I.P. (2008). Physiological of rabbilated graded levels of neem (Azadirachta indica) leaf meal. MSC Thesis Federal University of Technology, Owerri.
18) Paulino, I.C., Tsong, C.H., Strobe, B.E., Blasery M.J. (2006). Molecular analysis of fungal microbiota in sample from healthy humanis skin and psoriatic lessons. J. Clinical Microbiology 2006, 44, 2933-29.
19) Rabe, T. and Van Standen (1997). Isolation of an antimicrobial sesqui-ternoid from Worbugia alutaris. J. Ethanopharmcol 73:171-174.
20) Srivastava, R. (2000). Studying the information needs of medicinal plant stakeholders in Europe, TRAFFIC dispatches, 15:5.
21) Segre, J.A. (2006). Spideamal 142:885-892.barrier formation and recovery skin disorders. J. Clin. Invest. 2006. (111) 1150-1158.
22) Sharma, P. and Sharma, J.O. (2001). A review of plant species assessed in-vitro for anti-amoebic activity or both anti-amoebic and anti-plasmodial properties. Phytotherapy Research 15 (1): 1-16.
23) Till, A.G., Golden, V., Cunliffe, W.J., Holland, K.T. (2000). The cotaneousmicroflor of abodscout, resistant and lae-onset acne patient does not differ. Br. J. Dematology.
24) Treutlen, J., Smith, G.F.S., Van Wyk, B.E and Wink, W. (2003). Phylogenetic relationships in a sphodelecoal (alooideac) interned from chloroplast DNA sequence (RBOL, Malk) and from genomic fingerprinting (ISSR) Taxon 52:193.
25) Vasquez B., Avila, G., Segura, O. and Escalante, B. (1996). Antimicrobial and alkylating properties of 3-phosphonic derivatives of chromone. Pharmaceutical Medical Chemistry, 334:381-387.
26) Wang, H., Wany, T. (2004). (Determination of aloin content in callus of Aloe vera var. Chinansis) Zhang of Chinese medicinal material (in Chinese) 27(4);627-8 PMID 15 704580.
27) Yang, M.R., Kang, C.G., Roh, Y.S., Son, B.W., Choi, H.O., Park, Y.I., Lee, S.K., Choi, S.W. and Chung, M.H. (1998). The glycopeptides, a promoter of thymidine uptake from Aloe vera. Natural Product Science, 4(2);62-67.
28) Yates, A. (2002). Yates Garden Guide Harper Collins Australia. 2985-3123.