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Bitter Apple (*Citrullus colocynthis*): An Overview of Chemical Composition and Biomedical Potentials

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Abstract: The present study reveals the medicinal uses, chemical compositions and examines recent investigations on the therapeutic activities of extracts and chemicals identified from *Citrullus colocynthis*. The article also presents some of the functions of the chemicals present and attempt to emphasize and create an awareness of the great potential of *Citrullus colocynthis* as a source of novel agrochemicals, compounds of medicinal value and biologically active compounds.

Key words: Bitter apple, coastal sand dune, egusi

INTRODUCTION

Rapid industrialization and urbanization has led to the over-exploitation and loss of valuable neutral resources, including medicinally important herbaceous plants. Medicinal plants are in important source of compound for the pharmaceutical industry. They are many species of medicinal plant which are rare, endangered or threatened with extinction (Chand and Sahrawat, 2002). Many plants through out the World, including some with documented medicinal properties contain chemicals that are toxic to micro organisms. However, nearly 25% of modern medicines were described from plants that were first used traditionally (Hudson, 1989). The coastal areas in India, stretches over nearly 8000 km in length over Eastern and Western border. The coastal region forms the interface between terrestrial and marine ecosystem. The World Health Organization estimated that 80% of the people living in developing countries almost exclusively use traditional medicine.

Coastal sand dunes are mounds or ridges on the sand. They are formed by the sands deposited from the sub tidal and inter tidal regions. Wind is one of the most important factors, which helps in formation, movements and distribution of sand dunes. The sand dunes are colonized by vegetation beyond the highest high tide level. The sand dune vegetation is totally a different plant community with remarkable ability to locate hostile environment of drought, nutrient deficiency high winds, salts sprays and sand blast (Desai, 1995). The sand dune vegetation has a significant role to play in the coastal regions (Barson and Calder, 1981). It helps in prevention of sand erosion by decreasing wind speed at ground level. It stabilizes the dune habitat and migrates natural disturbances such as salt sprays, accretion wave action etc. In absence of vegetation sand moves from the beach to in land resulting desertification of inland. The coastal sand dunes are under threat due to natural disturbances and man made pressure. Main threats are (1) over exploitation of beach sands (2) indiscriminate cutting of coastal vegetation and (3) illegally uses of sand dunes for housing industries and other development activities. Therefore it is necessary to have strict and effective management polices for conservation and protection of sand dune vegetation. However coastal in India requires much more field and experimental data; in absence of which it is extremely difficult for further planning on management of sand dune ecosystem.

CHEMICAL COMPOSITION

The seeds are rich in fatty acids such as myristic, palmitic, stearic, oleic, linoleic and Linolenic acid. It is reported that the de-oiled cake can be incorporated in the cattle feed of milking cows up to 25% and it did not exhibit significant effect on the milk yield (Khatri *et al*., 1993). Tumba seed oil from is edible; its composition is similar to that of soybean oil. Refining and washing with citric acid removes its bitter taste (Ramakrishna *et al*., 1993).

Akhtar *et al*. (1999) reported that during germination of seeds in the dark at 30°C, the relative amounts of triacyl glycerol decreased, while the free fatty acids increased continuously in significant amounts. However, it was mentioned that saturated fatty acids are increased and unsaturated fatty acids decreased gradually during germination.

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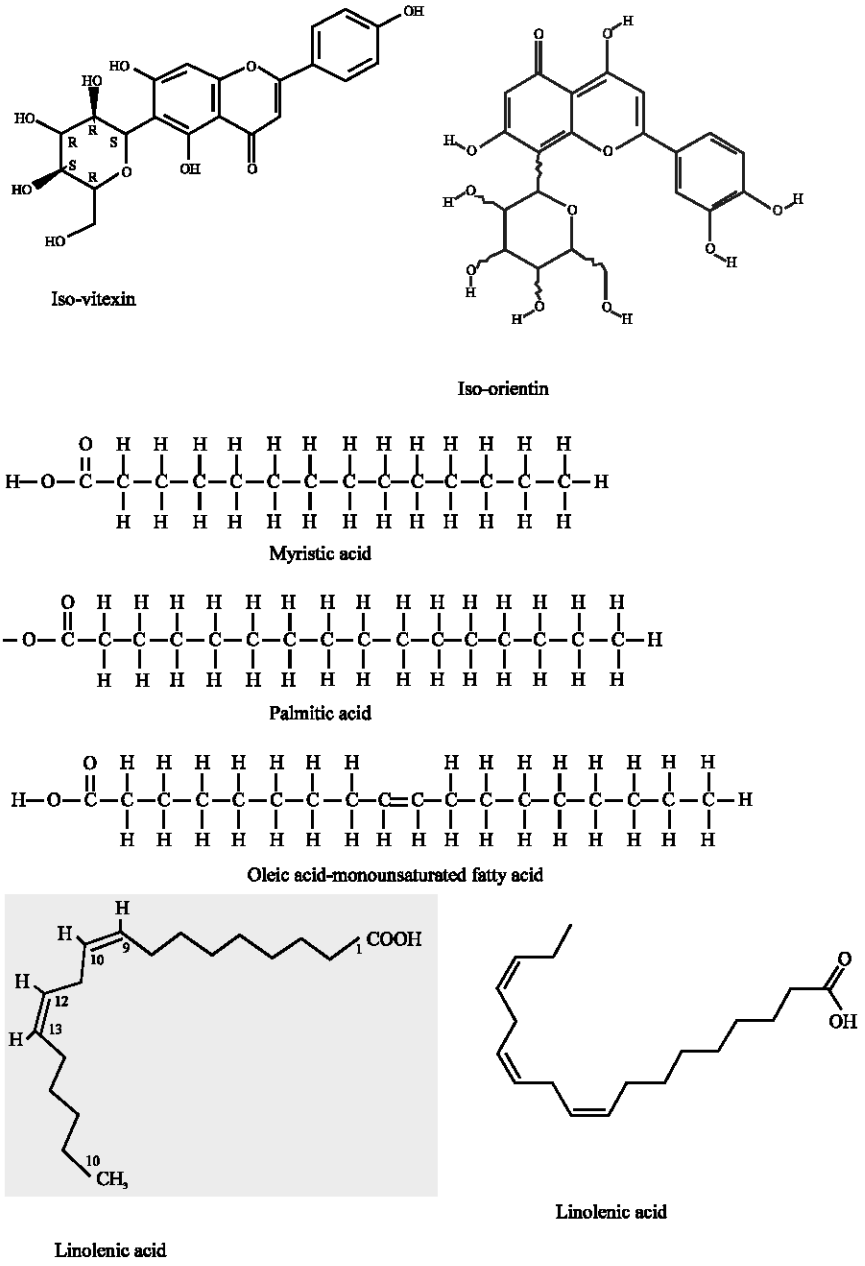

Fig. 1: Chemical constituents of *Citrullus colocynthis*

The protein content of seeds of colocynthis (transitional weed) was found to be 8.25% and rich in lysine, leucine and sulfo-amino acids viz., methionine. (Shaheen *et al*., 2003). Egusi (colocynthis) kernels contain oil (52%), protein (28.4%), fiber (2.7%), ash (3.6%) and carbohydrate (8.2%). These are good sources of essential amino acids (such as arginine, tryptophan and methionine) and vitamins (B1, B2, Niacin) and Minerals (Ca, Mg, Mn, K, P, Fe and Zn) (Simmons *et al*., 1982). Flavonoid quercetin was isolated from *in vivo* (leaf, stem, fruit and root) and *in vitro* callus of the species (Meena and Patni, 2008). Estimation of protein and amino acids composition of defatted seed meal showed high protein content (40.5%) and it was reported that the essential amino acid tryptophan is absent.

Flavone c-glucosides were identified from the fruits and aerial parts of *Citrullus colocynthis*. Fruit contains (Fig. 1) iso-vitexin, iso-orientin and iso-orientin 3'-methylether, while the aerial parts contain three C-p-hydroxy benzyl derivatives viz., 8-C-p-

hydroxybenzylisovitexin, 6-C-p-hydroxybenzylvitexin and 8-C-p hydroxybenzylisovitexin 4'-O-glucoside (Maatooq *et al*., 1997). The lipase and phospholipase extracted from the meal of mature seeds of *C. colocynthis* show optimum activity at 40°C and pH 7 in aqueous media. n-heptane was found to be the most suitable solvent medium to obtain maximum activity from these enzymes. The activity of lipase extracted from germinated seeds increases with the stage of development. However, the activity of phospholipase decreases with increase in size of the seeds (Akhtar *et al*., 1999).

Fruits of *Citrullus colocynthis* contains seventeen compounds were broadly identified and divided into five classes *viz*., alcohols, ketones, epoxy compounds, hydrocarbons and an acid. The alcohols identified were 4-(1-methyl) ethoxy, 1-Butanol; 5-methoxy, 2-methyl, 2-pentanol; 1-cyclopentyl, 2-propene-1-ol and 2-Furanmethanol, tetrahydro-5-methyl-*cis* and *trans* isomers. The ketones characterized were 3, 4-Dimethyl, 2-hexanone; 2-Methyl, 4-heptanone and 3-Methyl, 2-heptanone. Two epoxy compounds were 1-propoxy pentane and 2, 3-epoxy methyl propionate and palmitic acid. Four hydrocarbons might have been present on the surface the fruit in minimum quantities viz., tridecane, tetradecane, pentadecane and hexadecane. The two remaining compounds-one (viz., Trimethylsilylmethanol) impurity component derived from silicone oil used in the isolation process and the other impurity (viz., 1, 2-benzenedicarboxylicacid, diisooctylester) was stabilizer for plastics (Gurudeeban, 2007). All these compounds must have been derived by fatty acid pathway.

PROPERTIES OF EGUSI SEED PROTEINS

Egusi seed flours: Egusi seed flours had good quantities of sulfur, calcium, potassium, iron, magnesium, phosphorus and manganese. The mineral content was comparable to that of peanuts (Woodroof, 1969). The Total difference (TCD) values of 13.24 (a = 7.2, b = 8.4) and 13.93 (a = 1.4, b = 8.1) were obtained from the hull-free and 10% hull flours, respectively. Unsaturated fatty acids accounted for most of egusi seed oil with oleic and linoleic acids representing 15.9 and 62.81%, respectively. The saturated fatty acids were essentially palmitic (10.43%) and stearic (9.84%) acids. Higher carbon number fatty acids were not detected. (Girgis and Said, 1968). The essential amino acid that compare favorably with those of other oilseeds, especially sunflower and safflower meals (Sosulski and Sarwar, 1973). Unlike most oilseeds, egusi seed contains higher amounts of arginine, tryptophan and the sulfur containing amino acids.

Protein solubility, extractability and precipitability: Resuspension of the flour in 0.015 N NaOH (pH approximately at 10.5) readily ruptured the membrane and released all of the proteins. More different types of proteins in the alkaline soluble extracts are precipitated at pH 6.0 than 8.0. More water-soluble proteins are precipitated at pH 4.5 than 5.5. These results paralleled those of cottonseed water-soluble proteins precipitated at pH 4.0 and the alkaline-soluble components precipitated at pH 6.0, 5.0 and 4.0 (Akobundu *et al*., 1982).

Water and oil-holding capacity: The water-holding capacity for both hull-free and 10% hull-containing flours was 0.7 mL g^{-1}. The level of hull present in the flour had no influence on its water-holding capacity. The oil-holding capacity for the hull-free flour was 2.6 mL g^{-1} and that of the low-hull flour was slightly higher or 2.7 mL g^{-1} (Akobundu *et al*., 1982).

Emulsion capacity and viscosity: Basically suspensions of egusi seed flour formed two types of emulsions with in the pH range 2.5-10.5. Thin (salad dressing type) emulsions were formed at pH 2.5 and 5.0 (the latter was very thin). As the pH was increased from 6.5 to 10.5, progressively thicker (mayonnaise-type) emulsions were formed. At pH 5.0, which was around the isoelectric point of the proteins, very thin emulsions were formed with just 80 mL of oil. Thick emulsions formed at the point of high protein solubility (pH 10.5) and required 196 mL of oil. The viscosity of the foam was highest at pH 5.0 (Akobundu *et al*., 1982).

Foam capacity, stability and viscosity: Suspensions of 10% hull-containing egusi flour formed very thin foams between pH 5.0 and 6.7. Foams at other pH levels varied from thin at pH 2.5 to thick at 10.5. Foam volume increase was low between pH 5.0 and 6.7 but increased as more protein became soluble in the acid and alkaline pH ranges. Consequently, large amounts of foam were formed at pH's 2.5, 8.0 and 10.5. Although foam increase in pH 5.0 was the lowest, its stability was highest. In the alkaline pH region where more storage proteins were soluble, both foam capacity and stability were progressively enhanced. Similar observations were reported for cotton seed proteins.

Nutritional properties: The content of essential amino acids in the proteins of the flour makes it a good vegetable protein ingredient. The fiber in the 10% hull flour contributes nutritionally to it. Significant growth improvement was reported when egusi flour supplemented traditional West African diets either alone

or with other plant proteins (Sanchez *et al*., 1972). Feeding studies were not performed in this study but an investigation conducted elsewhere indicated that the biological indices of protein quality for egusi were lower than values obtained for soybean (Oyenuga and Fetuga, 1975). The first and second limiting amino acids in the flour are lysine and threonine, respectively. This partly supports the results of Oyenuga and Fetuga (1975) who noted that lysine and methionine were the first and second limiting amino acids, respectively of watermelon seeds, a close relative of egusi. Histidine has been known to be an essential amino acid for infants (Young and Scrimshaw, 1968) and the possibility that histidine is equally essential for a normal adult has also been suggested (Kopple and Swendseid, 1974). Thus the low content of histidine in egusi seed should be considered in the use of this product in food formulations, especially if the foods are intended for infants. Egusi seed flour contains several micronutrients (vitamins and minerals) that could contribute significantly to the diet. The potential for egusi seed flour as sources of calcium and niacin is encouraging to the low milk-consuming regions of lower West Africa where egusi cultivation thrives. These results further support the contention that ingredients of egusi seeds have the potential of being used in a number of food formulations.

Characteristics of crude oil: The freshly extracted oil was dark yellow in color with a greenish tint and had a mild odor and flavor. No extremely bitter taste in the oil was detected as was reported by Sayed *et al*. (1979) on the oil of *Citrullus colocynthis* in Egypt. The yield of oil extracted from *Citrulls colocynthis* whole seeds was found to be 24.86 and 26.1% on fresh and dry weight basis respectively. This is comparable to other oil seeds such as sunflower and safflower and in some what higher than that of cotton seeds and soybean (Swern, 1979). However, the yield of oil obtained here was much less than that obtained by Singh and Yadava, (1978) in India, who reported a fat content in *Citrullus colocynthis* seeds that ranged between 30.12-35.66% with a mean of 34.38%. The oil had a relatively high iodine value thus reflecting a high degree of unsaturation, a fair acid value and average values for the refractive index, unsaponifiable matter, Hehner number and Reichert-Meissl number when compared to other commonly consumed vegetable oils (Swern, 1979). TLC analysis of the crude oil showed that the major constituents were mainly the triglycerides (Rf = 0.74), free fatty acids (Rf = 0.37), phospholipids (Rf = 0.22) and sterols (Rf = 0.16) were also detected in addition to other minor undefined constituents. GLC analysis of the fatty acids methyl esters in conjunction with GLC-MS showed that the degree of unsaturation was over 75%. Linoleic acid was found to be the dominant fatty acid, 50.6% followed by oleic 25%, palmitic, 13.5%, stearic 10.5% and myristic acid 0.4% (Sawaya *et al*., 1983). The linoleic acid content was higher than that of cotton seed and close to that of sunflower, soybean and corn (Anonymous, 1961). There fore the *Citrullus* oil, like some other cucurbit seed oils might have a god potential use as a cooking oil in spite of the fact that the degree of unsaturation as well as its linoleic acid content are less than those of other known cucurbits seed oil such as *Cucurbita foetididsima* which was reported to have over 86% degree of unsaturation and 61-63% linoleic acid content (Bemis *et al*., 1975; Khoury *et al*., 1982). The absence of linolenic acid from the Citrullus oil might contribute positively to the stability of the oil upon storage.

Oxidative rancidity: Both the temperature and light had an adverse effect on the stability of the oil in terms of the formation of oxidative rancidity as evident from the higher peroxide values. Oil samples stored at a temperature of 32°C under both dark and indoor light conditions showed high peroxide values (5.5 and 7.6 meg kg^{-1}, respectively) than those stored at the lower temperature of 25°C (4.4 and 6.6 meg kg^{-1}), with the lowest values obtained for the oil samples stored at 7°C (1.0 meg kg^{-1}). However, at the end of the 5 months period of storage, no increase in the peroxide values was observed at 25°C for both samples stored in the dark and under indoor light conditions. Since, most consumable vegetable oil are stored either at room temperature, 20-25°C or even lower and usually in opaque containers, then the peroxide values obtained here for both groups, whether they were stored under indoor light conditions or in the dark, are still below the recommended standards of the Codex Alimentarius Commision (1970) for all the edible vegetable oils and hence can be relatively stable upon storage for at least several months.

PHARMACOLOGICAL STUDIES

Antimicrobial activity: *In vitro* antimicrobial activity was examined for aqueous and methanol extracts of *Citrullus colocynthis*. Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method (Bauer *et al*., 1960) against a number of antibiotics including two antifungal drugs. The agar disc diffusion method was followed for antibacterial susceptibility test. As a result of this study the aqueous extract showed high antibacterial activity against *E. coli* and *Staphylococcus aureus*, considerably less effect against *Klebseilla*

pneumoniae and *Bacillus subtillus* and in other, the aqueous extracts did not exhibit any antibacterial activity. On the other hand, methanol extracts of the plant showed high antibacterial activity against *Bacillus subtillis*, *Streptococcus pyogenes, Salmonella typhi*, considerably less activity against *Streptococcus faecalis* and other was no effect against *Proteus mirabilis*, *Proteus vulgaris* and *Vibro cholerae*.

Ethanol extracts of fruits, leaves, stem and roots were found to be active against Gram positive bacilli, viz., *Bacillus pumilus* and *Staphylococcus auerus*, while fruit and root extracts in double strength gave positive results against gram positive Bacillus subtilis. The gram negative bacilli viz., *Escherichia coli* and *Pseudomonas aeruginosac* showed no response (Memon *et al*., 2003). Antifungal activity was determined against six fungi. The stock culture was maintained in Glucose Peptone Yeast and Sucrose (GYPS) medium. The methanolic extract of the plant showed high antifungal activity against *Aspergillus fumigatus*, *Mucor* sp. and *Aspergillus flavus, Candida albicans, Pencillium* sp. and *Rhizopus* sp. did not show any antifungal activity (Gurudeeban *et al*., 2010).

Anti inflammation activity: The effect of *Citrullus colocynthis* leaf extract (CLEt) on acute inflammation using different inflammatory mediators-induced paw edema and sub acute inflammation (leukocyte infiltration and exudation) using carrageenan air-pouch model in rats. As a result CLEt significantly ($p<0.05$) inhibited carrageenan, serotonin and prostaglandin E1-induced paw edema. Maximum inhibition (48%) was found in prostaglandin E1-induced paw edema, whereas, it was 35% in carrageenan induced paw edema and 30.28% inhibition, respectively relative to vehicle treated control group. In the carrageenan air-pouch model CLEt and indomethacin significantly ($p<0.05$) reduced carrageenan-induced exudates volume and infiltration of neutrophils and monocytes into air-pouch compared to vehicle treated control group. We observed that CLEt at the doses of 250 and 500 mg kg^{-1} significantly inhibited carrageenan induced paw edema induced by prostaglandin E1 (48.56%) and serotonin (30.28%). These observations suggested that probably, anti inflammatory effect of *Citrullus colocynthis* not only by blocking the effects of serotonin and prostaglandin E1 on vascular membrane but also by inhibiting the release of these mediators (Rajamanickam *et al*., 2010). The anti inflammatory activity of CLEt extract found may be due to the presence of therapeutically active flavonoids such as apigenin, quercetin, naringein and luteolin (Khare, 2004). Flavonoids are known to prevent the synthesis of prostaglandins and have therapeutic application on inflammation (Havsteen, 2002).

Antidiabetic activity: The insulin secretory effects of these different extracts of *C. colocynthis* seed components were evaluated *in vitro* in the isolated rat pancreas and isolated rat islets in the presence of 8.3 mM glucose. All tested extracts, when perfused for 20 min at 0.1 mg mL^{-1}, immediately and significantly stimulated insulin secretion. The investigation revealed that different *Citrullus colocynthis* seed extracts have an insulin tropic effect which could at least partially account for the antidiabetic activities of these fruits (Nmila *et al*., 2000).

Jayaraman *et al*. (2009) speculate that the petroleum ether extract of *Citrullus colocynthis* fruits might be promising for the development of a standardized phytomedicine for the treatment of diabetes mellitus.

Acute toxicity studies revealed the non-toxic nature of the aqueous extract of *Citrullus colocynthis*. Oral administration of leaf suspension of *Citrullus colocynthis* (250 mg and 500 mg kg^{-1} b.wt.) for 60 days resulted in momentous reduction in blood glucose (from 381±34 to 105±35), glycosylated hemoglobin, a decrease in the activities of glucose-6-phosphatase and fructose 1, 6-bisphosphatase and an increase in the activity of liver hexokinase. The findings of study supported the anti diabetic asserts of *Citrullus colocynthis* (Gurudeeban and Ramanathan, 2010).

Anticancer activity: *In vitro* antioxidant activity in leaves and stem of *Citrullus colocynthis* evaluated. The enzymatic antioxidants such as catalase, super oxide dismutase, glutathaione reductase, glutathaione reductase and glutathiaone-S-transferase and non enzymic antioxidant (ascorbic acid, α-tocoperol, reduced glutathiaone, total carotenoids and flavonoids) were found to be present. The free radicals scavenging (DPPH, Hydroxyl radical and Nitric oxide) activity also observed. The study reported, *Citrullus colocynthis* might be a good source of natural antioxidant (Ramanathan *et al*., 2010b).

Citrullus colocynthis leaves were extracted resulting in the identification of cucurbitacin B/E glucosides. The cucurbitacin glucoside combination (1;1) inhibited growth of ER+ MCF-7 and ER-MDA-MB-231 human breast cancer cell lines. Cell-cycle analysis showed that treatment with isolated cucurbitacin glucoside combination resulted in the accumulation of cells at the G2/M phase of cell cycle. Treated cells showed rapid reduction in the level of the key protein complex necessary to the regulation of G2 exit and initiation of mitosis, namely the p34cdc2/cyclin B1 complex.

Table 1: Traditional uses, chemical constituents and scientific validation of *Citrullus colocynthis*

Plant parts	Chemical composition	Traditional Uses	Tested
Fruit	Iso-vitexin, iso-orientin and iso-orientin 3'-methylether 4-(1-methyl) ethoxy, 1-Butanol; 5-methoxy, 2-methyl, 2-pentanol; 1-cyclopentyl, 2-propene-1-ol and 2-Furanmethanol, tetrahydro-5-methyl- *cis* and *trans* isomers. 3, 4-Dimethyl, 2-hexanone; 2-Methyl, 4-heptanone and 3-Methyl, 2-heptanone, 1-propoxy pentane and 2, 3-epoxy methyl propionate and palmitic acid. tridecane, tetradecane, pentadecane and hexadecane	Anti pyretic, anthelmintic, tumor, bronchitis, asthma, tuberculosis, dyspepsia, constipation, anemia, throat diseases, elephantiasis	Antimicrobial studies, antidiabetic, anti inflammatory
Seed	Myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic	Antidiabetic	
Flour	Minerals: S, Ca, K,Mg, Sb, Sn, Si, Ag, Sr, Mb, Se, P, Fe, Zn, Cd, Cu, Ar, Co, Cr, Pb, Hg , Ni. Amino acids: Alanine, valine, glycine, isoleucine, leucine, praline, threonine, serine, methionine, phenyl alanine, aspartic acid, glutamic acid, tyrosine, lysine, histidine, argininme, tryptophan		
Leaves		Used for painful menstruation The leaves are diuretic and used in the treatment of asthma	Antimicrobial studies, antidiabetic, anti inflammatory, anti oxidant anti cancer, local anesthetic, mosquito larvicidal
Root		Inflammation of the breasts, amenorrhoea and rheumatism	

Cucurbitacin glucoside treatment also caused changes in the overall cell morphology from an elongated form to a round-shaped cell, which indicates that cucurbitacin treatment caused impairment of actin filament organization. This profound morphological change might also influence intracellular signaling by molecules such as PKB, resulting in inhibition in the transmission of survival signals. The treatment caused elevation in p-STAT3 and in p21WAF, proven to be a STAT3 positive target in absence of survival signals. Cucurbitacin glusoside treatment also induced apoptosis and changes in mitochondrial membrane potential. Cucurbitacin glucosides exhibit pleiotropic effects on cells, causing both cell cycle arrest and apoptosis. These results suggested that Cucurbitacin glucosides might have therapeutic value against breast cancer cells (Tannin-Spitza *et al*., 2007).

Local anesthetic activity: Local anesthetics are a well-known group of pharmaceutical agents used to relieve pain in specific parts of the organism, inhibiting propagation of signals along the nerves. The present study petroleum ether extract of *Citrullus colocynthis* was evaluated its local anesthetic activity in the animal frog *Rana hexadactyla*. Local anesthetics are intended to relieve pain by depressing or blocking the sensory nerves reversibly. Local anaesthetics block nerve conduction where applied locally to nerve tissue in appropriate concentration 1:10 and 1:100. They act on any part of the various systems and on every type of nerve fibre. Lumbar plexus anesthesia method (or) nerve block Anesthesia method is used to study the activity briefly. The study reported that the leaves are equally effective as that of the synthetic standard drug xyclocaine when placed in sciatic nerve. Further studies will be warranted to compare the mechanism of action (Ramanathan *et al*., 2010a).

Mosquito larvicidal activity: The larvicidal activity of crude acetone, hexane, ethyl acetate, methanol and petroleum ether extracts of the leaf of *Centella asiatica*, *Datura metal*, *Mukia scabrella*, *Toddalia asiatica*, extracts of whole plant of *Citrullus colocynthis* and *Sphaeranthus indicus* were assayed for their toxicity against the early fourth instars larvae of *Culex quinquefasciatus* (Diptera: Culicidae). However, the highest larval mortality was found in whole plant petroleum ether extract of *C. colocynthis*. Oleic and Linoleic acids were quite potent against fourth instar larvae of *Aedes aegypti* L. (LC50 8.80, 18.20 and LC90 35.39, 96.33 ppm), *Anopheles stephensi* Liston (LC50 9.79, 11.49 and LC90 37.42, 47.35 ppm) and *Culex quinquefasciatus* Say (LC50 7.66, 27.24 and LC90 30.71, 70.38 ppm) (Table 1) (Abdul Rahuman and Venkatesan, 2008).

Effect of *Citrullus colocynthis* on hair growth in albino rats: Roy *et al*. (2008) evaluated the petroleum ether and ethanol extracts of *C. colocynthis* on hair growth effect in albino rats. Minoxidil 2% solution was applied topically and served as the standard. Hair growth initiation time was significantly reduced to half on treatment with the petroleum ether extracts compared with untreated control animals. The treatment was successful in bringing a greater number of hair follicles (>70%) to anagenic phase than standard minoxidil (67%).

CONCLUSION

There is a great promise for development of novel drugs from the plant *Citrullus colocynthis* for dreadful human diseases, which have the potential drugs to treat.

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