

Anti-Cancer Studies on Semecarpus Anacardium with Special Emphasis to Angiogenesis

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Abstract: The project investigates the anti-angiogenesis activity of the semecarpus anacardium is widely used in traditional Ayurvedic medicines. For this investigation the semecarpus anacardium were dried and powdered. Preliminary phytochemical screening was performed using methanolic extract of dried powder. Different concentration (5mg, 10mg and 15mg) of the remaining dried powder is prepared. These preparations are added to the corresponding egg marked as 5mg, 10mg and 15mg respectively. Cinnamaldehyde is used as the positive control. These eggs were made to airtight by using tap. Three eggs were used for each sample and also three normal eggs were taken. These eggs were incubated for 21 days. After incubation period eggs were broken and observed for angiogenesis inhibition.

Keywords: anti cancer, semecarpus anacardium, cinnamaldehyde, Angiogenesis

1. Introduction

The Indian knowledge of herbal medicines is gaining widespread acceptance globally. In Ayurveda, almost all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures and so on (Farnsworth NR and Soejarto DD, 1991). In other parts of the world, the term Complementary and Alternative Medicine (CAM) is used for various forms of traditional drugs. Complementary and Alternative Medicine (CAM) can be defined as any treatment used in conjugation (complementary) or in place of (alternative) standard medical treatment. In alternative medicine, medicinal plant preparations have found widespread use particularly in the case of diseases not amenable to treatment by modern method (Dhalla S et al., 2006).

Semecarpus anacardium Linn. (Family: Anacardiaceae) is a plant well known for its medicinal value in Ayurvedic and Siddha system of medicine. Chemical and phytochemical analyses of its nut reveal the presence of biflavonoids, phenolic compounds, bhilawanols, minerals, vitamins and amino acids. A variety of nut extract preparations from this source are effective against many diseases, viz., arthritis, tumors, infections and so on. However, the mechanism of the pharmacological action of its nut can be greatly aided by the isolation of its active principle and determination of structure–function relationship.

Semecarpus anacardium is a deciduous tree. Like the closely related cashew, the fruit is composed of two parts, a reddish-orange accessory fruit and a black drupe that grows at the end. The nut is about 25 millimetres long, ovoid and smooth lustrous black. The accessory fruit is edible and sweet when ripe, but the black fruit is toxic and produces a severe allergic reaction if it is consumed or its resin comes in contact with the skin. The seed inside the black fruit, known as godambi is edible when properly prepared.

It is used for improving sexual power and increasing sperm count, curing diseases related to digestive system, balancing Kapha dosha in body. It is said that, no Kapha dosha remains after it is treated with this fruit. The redorange part is

collected and dried in sun. It is consumed after it is semi dried. If consumed in very large quantity, it is said to induce abortion. In moderation, it is however considered good for female reproductive system (Puri H. S, 2003).

Various parts of these plants are commonly used in the Ayurvedic system of medicine for the treatment of various ailments, mainly alimentary tract and certain dermatological conditions. Reports have shown noticeable impact on illnesses related to the heart, blood pressure, respiration, cancer and neurological disorders (Kleinsasser O,1988). The seed inside the *semecarpus anacardium* is known as "Godambi" in Hindi and is widely used in India as dry fruit. Godambi is eaten by Indians in winter and was commonly used as a method of birth control for women (Robin P.E, 1991).

Mineral Composition of Plant

The moisture content is low in all parts i.e. below 10, which is an indication that it will not be susceptible to microbial attack (Ogungbenle, 2006). The moisture content of the drug should not be too high thus it could discourage bacterial fungi or yeast growth. Equally important evaluation of crude drug is the ash value and acid insoluble ash value determination. The ash content in the plant parts ranged from 10-12% which indicates that high ash content i.e. 1030% shows presence of all the minerals and marks that the sample could be a better source of essential, valuable and useful minerals needed for good body development. Generally minerals from plant sources are less bio-available than those from animal sources. The extractive values are high in PE and NaOH compared to water. These are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Thomas *et al.*, 2008).

Alcohol has unique feature of dissolving all polar and all non polar constituents (Mukharjee, 2002). The ash value of the drug give an idea of the inorganic composition and other impurities present. The extractive values are used for determination of exhausted or adulterated drug (Singhal *et al.*, 2010).Elemental analysis reveals the presence of Sulphur (S),Calcium (Ca), Magnesium (Mg), Phosphorus (P) and Iron (Fe)in all parts of plants . Minerals play a very

important role in the plants. The plant is good source of inorganic minerals like S, Ca, Mg, P and Fe which are nutritionally important. Calcium plays dominant role in the maintaining the strength of stems. Calcium is important in blood clotting, muscle contraction and enzyme metabolism (de Oliveira *et al.*, 2001; Freitas *et al.*, 2002). The Nitrogen content ranges from 1.2 to 3.2% and is found high in roots and leaves. It is a precursor for amino acids. The crude powder of plant parts were analysed for the presence of heavy metals. Heavy metals Lead (Pb) and Mercury (Hg) were detected in traces i.e. ppm in leaves, stems and roots from 0.01-0.12 whose toxicity level was very low or negligible. The sample did not exceed the limit given according to the WHO guideline (WHO, 1998).

Lead was found to be 0.12 ppm and Mercury 0.07 ppm in roots. In leaves and stem, Pb was found to be 0.01 and 0.02 ppm and Hg was found to be 0.02 and 0.03 in leaves and stem respectively. It is believed that, presence of lead (Pb) is mainly due to the deposition or adsorption by their external parts.

The maximum limit of Pb is 10mg/Kg NMT 20PPM for Pb and Mercury (Shanthi *et al.*, 2010)

The amount of secondary metabolites in various parts of plant is presented in. The alkaloids content was very high in roots 3.18 mg/100gm while leaves contained 2.63 mg/100gm. Flavonoids are high i.e. 2.12 mg/100gm, 1.78 mg/100gm in roots and leaves respectively. The content of crude phenols was found high 3.22 mg/100gm in roots and 2.43 mg/100gm in leaves. The roots and leaves are rich in all the three. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic and antispasmodic and bacterial effects (Stray, 1998; Okwu and Okwu 2004). Flavonoids are potent water soluble anti oxidant and free radical scavengers which prevent oxidative cell damage have strong anti cancer activity (Salah *et al.*, 1995; Del-Rio *et al.*, 1997; Okwu, 2004). Flavonoids indicates anti inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties (Hodek *et al.*, 2002). The presence of phenols in plant indicates that the plant could act as anticlotting agent, antioxidant, immune enhancers and hormone modulator (Duke, 1992).

Toxicity evaluation

Semecarpus anacardium nuts can be given orally with milk, ghee, peanut oil etc. Toxic effect are not observed by such routes of administration. On the contrary, anabolic effects are obtained. Traditional methods recommended in Ayurveda and Siddha should be closely followed so as to get therapeutic effects without toxicity. Various reports have mentioned the range of dosage from 300 to 9000mg in graded manner. Toxicity studies were carried out by Ghosh *et al.* (1981), with one Siddha preparation of *semecarpus anacardium* (coded as SKx) and they found that, in rats, there was no adverse effect or mortality up to the oral dose of 2000mg/kg. The histopathological studies on liver, lung, kidney and heart did not reveal any significant pathological lesions even when the extract was administered at a high dose of 1000mg/kg. The animals looked healthy and active without any physiological disturbance and loss in body

weight. Hematological picture was almost normal. The extract did affect total WBC count but there was no effect on RBC count and hemoglobin percentage.

The toxic- side effects of the high dose of the drug are diarrhea and vomiting, swelling all over the body, ulceration and vesication on the skin. It should be used cautiously and in lesser doses in hot season. During use, whether external or internal the least appearance of rash or redness of the skin or an itchy or uneasy sensation in any part of the body should be considered as a manifestation of undesirable effects and use should be discontinued immediately.

Angiogenesis

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. This distinct from vasculogenesis, which is the de novo formation of endothelial cells from mesoderm cell precursors. The first vessels in the developing embryo form through vasculogenesis, after which angiogenesis is responsible for most, if not all, blood vessel growth during development and in disease.

Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in the formation of granulation tissue. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer. The essential role of angiogenesis in tumor growth was first proposed in 1971 by Judah Folkman, who described tumors as "hot and bloody".

Tumor angiogenesis

Cancer cells are cells that have lost their ability to divide in a controlled fashion. A malignant tumor consists of a population of rapidly dividing and growing cancer cells that progressively accrues mutations. However, tumors need a dedicated blood supply to provide the oxygen and other essential nutrients they require in order to grow beyond a certain size (generally $1-2 \text{ mm}^3$).

Tumors induce blood vessel growth (angiogenesis) by secreting various growth factors (e.g. VEGF). Growth factors such as bFGF and VEGF can induce capillary growth into the tumor, which some researchers suspect supply required nutrients, allowing for tumor expansion. Unlike normal blood vessels, tumor blood vessels are dilated with an irregular shape. In 2007, it was discovered that cancerous ones), PKC apparently limits beta-catenin, which solicits angiogenesis. Other clinicians believe angiogenesis really serves as a waste pathway, taking away the biological end products secreted by rapidly dividing cancer cells. In either case, angiogenesis is a necessary and required step for transition from a small harmless cluster of cells, often said to be about the size of the metal ball at the end of a ball-point pen, to a larger tumor. Angiogenesis is also required for the spread of a tumor, or metastasis. Single cancer cells can break away they can implant and begin the growth of a secondary tumor. Evidence now suggests the blood vessel in a given solid tumor may, in fact, be mosaic vessels, composed of endothelial cells and tumor cells. This mosaicity allows for substantial shedding of tumor cells into the vasculature, possibly contributing to the appearance of

circulating tumor cells in the peripheral blood of patients with malignancies. The subsequent growth of such metastases will also require a supply of nutrients and oxygen and a waste disposal pathway.

Endothelial cells have long been considered genetically more stable than cancer cells. This genomic stability confers an advantage to targeting endothelial cells using anti angiogenic therapy, compared to chemotherapy directed at cancer cells, which rapidly mutate and acquire 'drug resistance' to treatment. For this reason, endothelial cells are thought to be an ideal target for therapies directed against them however, that endothelial cells growing within tumors do carry genetic abnormalities. Thus, tumor vessels have the theoretical potential for developing acquired resistance to drugs. This is a new area of angiogenesis research being activity pursued.

Two independent studies published in the journal Nature in 2010 November confirmed the ability of tumors to make their own blood vessels. When one group found that tumor stem cells could make their own blood vessels and avastin could not inhibit their early differentiation, the other group showed that selective targeting of endothelial cells generated by tumor derived stem cells in mouse xenografts resulted in tumour reduction. These studies done in glioblastoma model may have implications in other tumors.

Formation of tumor blood vessels.

Angiogenesis research is a cutting-edge field in cancer research, and recent evidence also suggests traditional therapies, such as radiation therapy, any actually work in part targeting the genomically stable endothelial cell compartment, rather than the genomically unstable tumor cell compartment. New blood vessel formation is a relatively fragile process, subject to disruptive interference at several levels. In short, the therapy is the selection process, subject to disruptive interference at several levels. In short, the therapy is the selection agent which is being used to kill a cell compartment. Tumor cells evolve resistance rapidly due to rapid generation time (days) and genomic instability (variation), whereas endothelial cells are a good target because of a long generation time (months) and genomic stability (low variation). This is an example of selection in action at the cellular level, using a selection pressure to target and differentiate between varying populations of cells. The end result is the extinction of one species or population of cells (endothelial cells), followed by the collapse of the ecosystem (the tumor) due either to nutrient deprivation or self-pollution from the destruction of necessary waste pathways.

Angiogenesis-based tumor therapy relied on natural and synthetic angiogenesis inhibitors like angiostatin, endostatin and tumstatin. These are proteins that mainly originate as specific fragments of pre existing structural proteins like collagen or plasminogen.

Recently, the first FDA-approved therapy targeted at angiogenesis in cancer came on the market in the US. This is monoclonal antibody directed against an isoform of VEGF. The commercial name of this antibody is Avastin, and the therapy has been approved for use in colorectal cancer in

combination with established chemotherapy. (www.canceractive.com)

| | |
|-----------------|-----------------------|
| Kingdom: | Plantae |
| (Unranked) : | Angiosperms |
| (Unranked) : | Eudicots |
| (Unranked) : | Rosids |
| Order: | Sapindales |
| Family: | Anacardiaceae |
| Genus: | Semecarpus |
| Species: | S. anacardium |
| Binomial Name : | Semecarpus Anacardium |

Sample Description



Semecarpus anacardium is an average growing tree normally upto 10-15m high and the bark is dark grey in colour which produces an irritating substance.

The leaves of this plant are 30-60cm long and 12-30cm wide, glabrous and pubescent. The flowers produces are green – white and fruit is 2-3cm broad, ovate, smooth berry which turns black after ripening (Govindachary TR et al.,1971).

Chemical Composition of Nuts:

Anacardic acid cardol, catechol anacardol fined oil, sernicarpoiadbhilawanol.

Dosage:

Detoxified fruit – 1 – 2g is milk confection.

Side effects of warning:

- *Semecarpus anacardium* is used in ayurveda after proper purification. The nuts should not be used as home remedy. The seeds are hot in potency that gives heating effect to body. Therefore it should not be used in hot weather. It should not be used by kids and pregnant women.
- The nuts has anti-fertility effect.
- People with excess bile should not use it.

2. Aim and Objectives

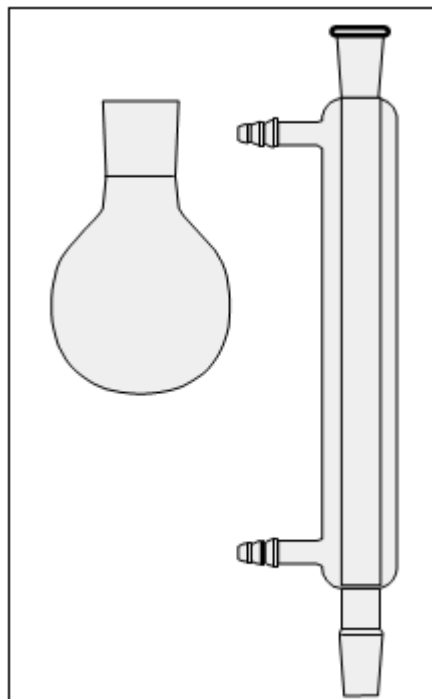
Aim

To find out the anti-angiogenesis activity of *Semecarpus anarcadium* seed methanolic extract using chick embryo method.

Objectives

- Collection of the seed of *Semecarpus anarcadium*.
- Collection of fertilized eggs.
- Grinding and drying of the seed .
- Extraction and preliminary phytochemical screening of the dried sample (10-15g)
- Preparation of different concentration of the remaining dried material.
- Application of the different concentration of to the eggs.
- Application of the drug cinnamadehyde as a positive control to egg.
- Incubate for 21 days
- Cutting of the egg and take photographs

3. Materials and Methods



Refluxing

- Take shade dried and powdered material around 5-10 gm and weigh accurately
- Take a 250 ml round bottom flask with condenser
- Put the dried material into the flask and add 100 ml of the solvent
- Add 3-4 glass beads for avoiding the bumping of the solvent
- The flask is then place in a mantle and connect the condenser
- Connect the rubber tubings to the condenser and allow the water to flow from bottom to top.
- Switch on the mantle after ensuring the continuous flow of water

- Adjust the temperature according to the boiling point of the solvent being used.
- Let the process continue for 3 hours.
- After three hour collect the extract alone from the flask by decanting and add 100 ml fresh solvent and again start heating.
- Process continue for 2 hours and collect the extract and add to the first
- 100 ml
- Again add 100 ml of the solvent to the flask and boil again for 2 hours
- Club all the three extracts and concentrate to 50 ml
- Filter the extract through wattman filter paper and store in a 50 ml standard flask.
- The 50 ml extract can be used for quantitative purpose.

Preliminary Phytochemical Screening of the Plant

The methanol, benzene and milk extracts are used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

- Molisch's test: Dissolved small quantity of 300mg alcoholic and dried seed extract powder of semecarpus anarcadium separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.
- Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.
- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.

Test for flavanoids

- Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drug.
- Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

- Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid

- Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

- Dragendroff’s test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified
- Dragendroff’s reagent. Orange coloration of the spot indicated the presence of alkaloids.
- Hager’s test: The extract was treated with few ml of Hager’s reagent. Yellow precipitation indicated the presence of alkaloids.
- Wagner’s test: The extract was treated with few ml of Wagner’s reagent. The reddish brown precipitation indicated the presence of alkaloids.
- Tests for Glycosides.
- Legal’s test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

- Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes. A 1cm layer of foam formation indicates the presence of Saponin.

Test for Anthraquinones

- Borntrager’s test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

- Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

| Compound | Tests performed | Observations | | |
|---------------|--------------------------------|--------------|------|---------|
| | | Methanol | Milk | Benzene |
| Carbohydrates | Molisch’s test | - | - | - |
| | Fehling’s test | - | - | - |
| Phenols | Phosphomolybdic acid test | +++ | +++ | + |
| Flavonoids | Shinoda test Lead acetate test | +++ | +++ | + |
| Tannins | Braemer’s test | - | - | - |
| Alkaloids | Dragendroff’s test | ++ | +++ | ++ |
| Terpenes | Liebermann-Burchardt test | - | - | - |
| Glycosides | Legal’s test | - | - | - |
| | Borntranger’s test | - | - | - |
| Saponins | Foam test | - | - | - |
| Sterols | Salkowski’s test | - | - | - |
| Aminoacids | Ninhydrin test | - | - | - |

Anti-Angio-Genesis

- Fertilizing eggs in triplicates were used for the assay.
- The embryo side was cut using a surgical blade.
- Positive control were used as Cinnamaldehyde 5mg
- The extracts were induced to the drugs using micro-syringe.

- After the application of the drug the cover was repositioned using bandages
- The eggs were then subjected to incubation for 21 days.
- After 21 days the egg was opened and photographs were taken

4. Result and Discussion

Preliminary Phytochemical screening of *Semecarpus anarcadium*

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

Test for fixed oils and fats

- Press small quantity of the petroleum ether extract between two filter paper. Oil stains on the paper indicated the presence of fixed oils.

Note: the results for the above experiments can be noted as follows.

ANTI-ANGIO-GENESIS

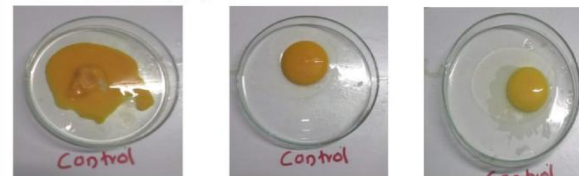


Figure - 2 : Normal egg



Cinnamaldehyde

Cinnamaldehyde

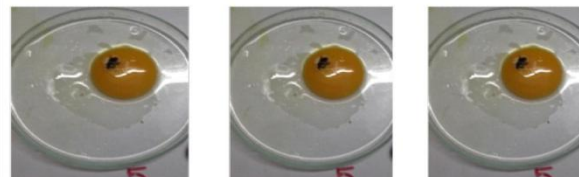
Cinnamaldehyde



5 mg Methanol

5 mg Methanol

5 mg Methanol



5 mg Milk

5 mg Milk

5 mg Milk

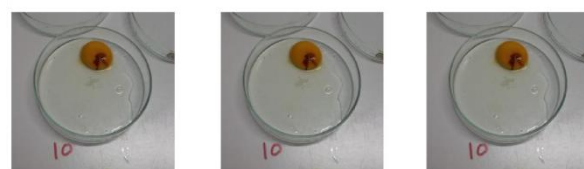
Figure - 5



5mg Benzene
Figure - 6



10 mg Methanol
Figure - 7



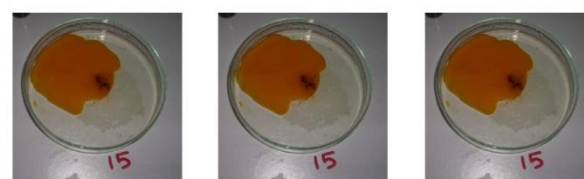
10 mg Milk
Figure - 8



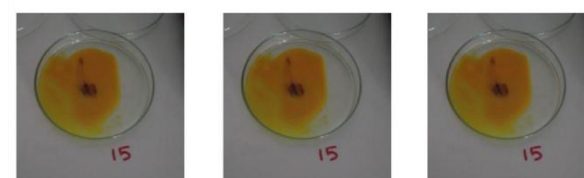
10 mg Benzene
Figure - 9



15 mg Methanol
Figure - 10



15 mg Milk
Figure - 11



15 mg Benzene
Figure - 12

5. Summary and Conclusion

The present scientific investigation deals with the identification of class of chemical compound present in the different extracts of *semecarpus anacardium*.

The current work reveals that the alkaloids are the major component present in the seed of *semecarpus anacardium*.

The anti-cancer activity was prepared using anti-angiogenesis method and the positive control used as cinnamaldehyde. The work revealed that the seed of *semecarpus anacardium* which was treated with the cow milk showed the better activity when compared to the other.

Then we conclude that the seed of *semecarpus anacardium* is highly potent in term of anti-cancer activity with specific emphasis to antiangiogenesis activity. The seed which are treated with milk showed significant activity. The identification of chemical compound which are responsible for the specific activity is remaining in this work. The identification of novel anti-cancer compound will be a boon for the entire pharmaceutical industry.

References

- [1] A.D.Naveen Kumar, Ganesh BabuBevara, antioxidant, cytoprotective and antiinflammatory activities of stem bark extract of *semecarpus anacardium*, asian journal of pharmaceutical and clinical research,6(2010),PP.26-58
- [2] Alzoreky NS, Nakahara K Synthesis of gold nanoparticles by various leaf fractions of *Semecarpusanacardium* L. treecommonly in Asia. Int. J. FoodMicrobiol,4(2003),pp. 223–230.
- [3] B Premalatha, P Sachdanandam Modulating role of *Semecarpusanacardium*L. nut milk extract in aflatoxin B₁biotransformation Pharmacol Res, 41 (2000), pp. 19–24
- [4] B Premalatha, P SachdanandamImmunomodulatory activity of *Semecarpusanacardium*Linn.nut milk extract in aflatoxin B₁induced hepatocellular carcinoma Pharm PharmacolCommun, 4 (1998), pp. 161-166
- [5] B Premalatha, V Sujatha, P Sachdanandam Modulating effect of *Semecarpusanacardium*Linn.nut extract on glucose metabolizing enzymes in aflatoxin B₁induced experimental hepatocellular carcinoma,Pharmacol Res, 36 (1997), pp. 187–193
- [6] B Premalatha,PSachdanandam,*Semecarpus anacardium* Linn. Nut Milk Extract in Aflatoxin B₁-Induced Hepatocellular Carcinoma, Journal of Clinical Biochemistry and Nutrition, 25 (1998) , PP 63-70
- [7] Chopra RN. 2nd ed. Calcutta: Academic Publishers; 1982.
- [8] Indigenous drugs
- [9] D Das,K.Wood,anatomy of some timbers of Anacardiaceae of Bangladesh Bulletin - Wood Anatomy Series, Forest Research Institute (Chittagong),54(1984), pp.31-52
- [10] De'Oliveira, A.C., Perez, A.C., Merino, G., Prieto, J.G. andAlvarez, A.I. 2001. Protective effects of *Panax ginseng* on muscle injury and inflammation after eccentric exercise. *Comp. Biochem. And Physiol.* 130c: 369-377.
- [11] Del-Rio A., Obdulio, B.G., Casfillo, J., Marin, F.G. and Ortuno,A. 1997. Uses and Properties of citrus flavonoids. *J. AgricFood Chem.* 45: 4504-4515.

- [12] Dhalla S, Chan KJ, Montaner JS, Hogg RS. Complementary and alternative medicine use in British Columbia: A survey of HIV positive people on antiretroviral therapy. *Complement Ther Clin Pract.* 2006; 12:242–8. [PubMed]
- [13] Duke, J. 1992. *Handbook of Biological Active Phytochemicals and their Activities*. BOCA Ration (FL) CRC Press, 99-131.
- [14] Farnsworth NR, Soejarto DD. Global importance of medicinal plants. In: Akerele O, Heywood V, Syngé H, editors. *Conserv Med Plants*. New York: Cambridge University Press; 1991. pp. 25–51.
- [15] Freitas, A.M., Schor, N. and Boim, M.A. 2002. The effect of *Phyllanthusnurun* urinary inhibitor of calcium oxalateCrystallization and other factors associated with renal stoneformation. *BJU int.* 89 (9): 829-834.
- [16] Ghosh D, Thejomoorthy P, Shetty BMV & Veluchamy G, Certain pharmacological studies with SKx (a coded anti-cancer siddha preparation) with special reference to its toxicity, *J Res Ayur Siddha*, (1981), 150.
- [17] Govindachary TR, Joshi BS, Kamal VM. Phenolic constituents of *Semecarpus anacardium*. *Indian J Chem.* 1971;9:1044.
- [18] Hodek, P., Thefil, P. and Stiborova, M. 2004. Flavonoids-Potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico-Biol. Int.* 139(1): 1-21.
- [19] Kadam, R. M. Allapure, Studies on antifungal properties of essential oil of *Semecarpus anacardium* against seed mycoflora, *International Journal of Plant Protection*, 2(2009), pp. 126-127
- [20] Karthikayan, A., Shanthi, V. and Nagasathaya, A. 2009. Preliminary phytochemical and Antibacterial screening of crude extract of the leaf of *Adhatodavasisal*. *Int. Jour. Green Pharm.* 3:78-80.
- [21] Kleinsasser O., *Tumors of the Larynx and Hypopharynx*, Georg Thieme Verlag, Stuttgart, 1988.
- [22] KR Kirthikar, BD Basu Separation of the bhlawanols A and B and a comparative study of their growth inhibitory effect on Clostridium tetani and general pharmacology,, *Bulletin of Haffkine*
- [23] Institute, 9India (1933), pp. 667–680
- [24] Kumar, B. H. Thakur, Certain non-edible seed oils as feeding deterrents against Spodopteralitura Fb, *Journal of the Oil Technologists' Association of India*, 20(1988), pp. 63-65
- [25] Mohmad Vasim Sheikha, Navin Devadiga and Dr. Manish Hate, *comparative studies of in-vitro anti-oxidant and anti-inflammatory potential of semecarpus ancardium* linn. f leaves, *world journal of pharmacy and pharmaceutical sciences*, 4(2011), pp.78102
- [26] Mukherjee, P.K. 2002 Quality control of herbal drugs: An approach to evaluation of botanicals. *Bussiness Horizons*, New Delhi. 362.
- [27] NS Prakasha Rao, L Ramachandra Rao, RT Brown, Antimicrobial efficacy of nut oil of *Semecarpus anacardium*, *Phytochemistry*, 12 (1973), pp. 671-682
- [28] Ogungbenle, H.N. 2006 Chemistry, functional properties of some edible oil seeds. *La Rivista Italiana*. Vol. LXXX III, 81-86.
- [29] Okwu, D.E. and Okwu, M.E. 2004. Chemical composition of *Spondias mombin* Linn plant parts. *J. Sustain Agric. Environ.* 6(2): 140-147.
- [30] P Vijayakumar, N Subramanian, Neuroprotective effect of *Semecarpus anacardium* against hyperammonemia in rats, *Journal of Pharmacy Res*, 3(2010), pp. 145-156
- [31] Parag A. Pednekar, Bhanu Raman, I, assessment of *semecarpus anacardium* (linn.f.) leaf methanolic extract for their antibacterial, antifungal and antioxidant activity, *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(1995), pp. 75-90
- [32] Parag A. Pednekar, Bhanu Ramani, pharmacognostic and phytochemical studies of *semecarpus anacardium* (linn.f.) leaves, *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(1991), pp. 104-112
- [33] Plouvier, V Surla, recherche des acides quiniques et shikimiques chez quelques Anacardiacees, *Compte Rendu Hebdomadaire des Seances de l'Academie des Sciences*, 50(1960), pp. 1721
- [34] Puri, G. S. Gupta, the calcium content of the foliage of Sal and its common associates in the Dun valley. *Journal of the Indian Botanical Society* 29(1950), pp. 139-144
- [35] Puri, H. S. (2003) *RASAYAN: Ayurvedic Herbs for Longevity and Rejuvenation*. Taylor & Francis, London, 8(2003), pp. 74-79
- [36] Robin P.E., Reid A., Powell D.J. and McConkey C.C., The Incidence of Cancer of the Larynx, *Clinotolarygol*, 1991, 16, 198-201.
- [37] S Angsubhakorn, P Get Ngern, M Miyamoto, N Bharamarpravati Seed composition and fatty acid profile of some tree borne oilseeds, *Int J Cancer*, 46 (1990), pp. 664–668
- [38] S.N Murthy, *Anticancerous efficacy of ayurvedic milk extract of Semecarpus anacardium nuts on hepatocellular carcinoma in wistar rats*, *Journal of Chemistry*, 228 (1983), pp. 1167–1172
- [39] Salah, N., Miller; N.J., Pagange, G., Tijburg, L., Bolwell, G.P., Rice, E. and Evans, C. 1995. Polyphenolic flavonols as scavengers of aqueous phase radicals as chain breaking antioxidant. *Arch Biochem. Biophys.* 2:339-346.
- [40] Singhal, A.K., Bhati, V.S. and Singhal, V.K. 2010 Pharmacognostic study of aerial parts of plants *Geniosporum prostratum* (L.) Benth. *Jour. Sci. Specul. Res.* 1(1):19-24.
- [41] Stray, F. 1998. *The Natural Guide to Medicinal Herbs and Plants*. Tiger Books International, London: 12-16.
- [42] T Vijayalakshmi, VMuthulakshmi, P Sachdanadam Effect of milk extract of *semecarpus anacardium* nuts on glycohydrolases and lysosomal stability in adjuvant arthritis in rats, *journal of Ethnopharm*, 13(1997), pp. 1-8
- [43] Tereza Dantas, Natália Freitas Oliveira, Rosélia Sousa Leal, The importance of the cashew nut (*Anacardium occidentale* L.) coat: a review, *American International Journal of Contemporary Scientific Research*, 8(1990), pp. 258-275
- [44] Thomas, S., Patil, D.A., Patil, A.G. and Chandra, N 2008 Pharmacognostic evaluation and World Health Organization 1998. *Quality control methods for medicinal plant materials*, Geneva, Switzerland.

- [45] V. N. Bondre and V. N. Nathar, phytoconstituents, proximate and mineral composition of *semecarpusanacardium* an ethnomedicinal plant, Department of Botany, SantGadge Baba Amravati University,
- [46] Amravati 6(2011), pp.423-428
- [47] V.Kadam, Determination of ash values of three endangered medicinal taxa of South Gujarat Forest, Plant Archives9(2009) , pp. 27-29
- [48] VS Aboobaker, AD Balgi, RK Bhattachariya Urushiol-induced contact dermatitis caused during Shodhana (purificatory measures) of Bhallataka (*Semecarpus anacardium* Linn.) fruit, An international Quarterly journal of Research in Ayurveda, 8 (1994), pp. 1095–1098
- [49] World Health Organization 1998. *Quality control methods for medicinal plant materials*, Geneva, Switzerland.
- [50] Y. Sushma, Gajanana Kulkarni and Shilender Singh, antifertility activity of aqueous and ethanolic extracts of *semecarpusanacardium* fruit in female albino rats Department of Pharmacology, Subbhaiah Institute of Medical Sciences and Research Centre, Shivamogga, Karnataka, 7(2012), pp.1235-1242.