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Evaluation and Hepatoprotective Activity of the Aqueous Extract of *Aegle Marmelos*

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INTRODUCTION:

Herbal remedies have a therapeutic effect and are acceptable interventions for diseases and symptoms. Interestingly, demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries (Abere *et al.*, 2010). The World Health Organisation (WHO) estimates that about 80% of the developing world‘s population meets their primary healthcare needs through traditional medicine (Abere *et al.*, 2010; Calixto, 2000; Green, 2000; Jadeja *et al.*, 2011). About 25% of drugs prescribed and dispensed in the United States contain at least one active component derived from plant matter.

Aegle marmelos

Botanical details

A. marmelos is a medium sized, slow growing tree, 25-30 feet tall. The stem is short, thick, soft, flaking bark, and spreading, occasionally spiny branches, the lower ones are floppy. Young suckers put up with many rigid, straight spines. There are pointed, axial one inch long spikes on this tree. The leaflets are oval or lancet shaped, 4-10 cm long, 2-5cm wide. Leaves composed of 3-5 leaflets in it. The lateral leaflets are lacking petiole and the terminal one has an elongated one. The petiole is 1 to 2.5 inch long. Mature leaves emit a peculiar fragrance when bruised. Flowers occurs in clusters of 4 to 7 along the young branchlets, have 4 recurved, fleshy petals. The flowers are greenish white in color with a peculiar fragrant. Flowering occurs during the month of May and June. Fruit is spherical or oval in shape with a diameter of 2 to 4 inch. Shell is

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thin, hard and woody in nature. It is greenish when unripe and upon ripening it turns into yellowish color. The pulp of the fruit has 8-15 segments. The pulp is soft, pasty, sweet, yellow, resinous and fragrant. Fruition occurs in the month of May and June. The seeds are embedded in the pulp. The seeds are small (nearly 1 cm in length), hard, bearing woolly hairs, flattened-oblong, and each enclosed in a sac of adhesive (Lambole et al., 2010).

Botanical classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta
Super division	:	Spermatophyta
Subclass	:	Rosidae
Class	:	Sapindales
Order	:	Fabales
Family	:	Rutaceae
Genus	:	*Aegle* Corr. Serr.
Species	:	*Aegle marmelos* (L.) Corr. Serr.

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Fig. 1: Fruit of *A. marmelos*

Fig. 2: Leaves of *A. marmelos*

Fig. 3: Tree of *A. marmelos*

Fig. 4: *Flowers* of *A. marmelos*

Traditional uses:

A. marmelos is extensively described in the Vedic literature for the treatment of various diseases. *A. marmelos* is traditionally used to treat dysentery, constipation, jaundice, stomachache, stomachic, fever, chronic diarrhea, asthma, febrile delirium, acute bronchitis, inflammations, acidity, burning sensation, epilepsy, indigestion, leporsy, snakebite, ulcers, mental illnesses, nausea, smallpox, spermatorrhoea, leucoderma, abdominal discomfort, myalgia, eye disorders,

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sores, swelling, thyroid disorders, tumors, ulcers thirst, and upper respiratory tract infections (Sekar et al., 2011).

Phytochemistry

The leaves of *A. marmelos* contain various types of secondary metabolites namely cardiac glycosides, flavonoids, alkaloids, terpenoids, tannins, saponins and steroids (Venkatesan et al., 2009; Sivaraj et al., 2011). The fruit pulp of *Aegle marmelos* are rich in phytoconstituents containing all the chemical constituents present in leaves. Additionally, calcium, fiber, fat, potassium, Iron, phosphorus, minerals and vitamins (vitamin A, vitamin B1, vitamin C and Riboflavin), lignin, fat and inulin are present in the fruit pulp (Rajan et al., 2011).

REVIEW OF LITERATURE:

Karmase et al., 2013 reported that adipogenesis inhibition by *A. marmelos* as one of the path for its anti-obesity effect. *A. marmelos* induced anti-nociception is mediated through both opioid and monoaminergic pain pathways; suggest its possible use in chronic pain (Kothari et al., 2013). The aegelinosides A and B, anhydromarmeline isolated from the *Aegle marmelos* leaves as α-glucosidase inhibitors (Phuwapraisirisan et al., 2008). The methanol extract of *A. marmelos* leaves demonstrated significant analgesic activity in mice (Shankarananth et al., 2007).

Nugroho et al., 2013 determined the affinity of six active compounds of *Aegle marmelos* Correa, they are (S)-aegeline, aurapten, zeorin, (E, R)-Marmin, skimmianine, and dustanin as antihistamines in histamine H1 receptor. Based on molecular docking, Amino acid residues involved in ligand protein interactions were Lys179, Asp107, Asn198, Lys191, and Trp428. *Aegle marmelos* possess potential anxiolytic and antidepressant activities and it enhances the anxiolytic and antidepressant activities of imipramine and fluoxetine (Kothari et al., 2010).

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Das and Roy C, 2012 reported that Pretreatment with *Aegle marmelos* fruit pulp extract for 14 consecutive days in experimental animals showed the reverse effects of aspirin suggesting gastro-duodenal protective and anti-ulcerogenic properties of *Aegle marmelos* through its antioxidant mechanism. Mazumder et al., 2006 established the efficacy of *Aegle marmelos* (Correa) Linn. root extract as an effective anti-diarrhoeal agent. The methanol extract of *A. marmelos* exhibited significant immunomodulatory activity by exciting cellular and humoral immune mechanisms (Govinda and Asdaq, 2011).

Nugroho et al., 2011 concluded that marmin, a compound isolated from *A. marmelos* could inhibit contraction of the guinea-pig tracheal smooth muscle, especially by interfering histamine receptor, inhibiting the histamine release from mast, inhibiting intracellular Ca^{2+} release from the intracellular store and the Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The aegeline possessed significant antihistamine activity by inhibiting the mast cell. *A. marmelos* evaluated for hepatoprotective activity, toxicity, tumor promotion and subsequent cell proliferation response in Wistar rats, and it demonstrated the significant lowering capacity.

Drug Metabolism in liver

The ability of humans to metabolize and clear drugs is a natural process that involves the same enzymatic pathways and transport systems that are utilized for normal metabolism of dietary constituents. The human body identifies almost all drugs as foreign substances (xenobiotic) and subjects them to various chemical processes to make them suitable for elimination. This involves chemical transformations to reduce fat solubility and to change biological activity. Although almost all tissues in the body have some ability to metabolise chemicals, smooth endoplasmic reticulum in the liver is the principal metabolic clearing house for both endogenous chemicals (example, fatty acids, and steroid hormones) and exogenous substances like drugs (Blumenthal *et al.*, 2006).

Drug metabolism is usually divided into two phases: phase 1 and phase 2. Phase 1 reaction, in which enzymes carry out oxidation, reduction, or hydrolytic reactions, is thought to prepare a

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drug for phase 2 in which enzymes form a conjugate of the substrate (the phase 1 product). These processes tend to increase water solubility of the drug and can generate metabolites which are more chemically active and potentially toxic. Most of phase 2 reactions take place in the cytosol and involve conjugation with endogenous compounds via transferase enzymes. Chemically active products from phase 1 are made relatively inert and suitable for elimination by the phase 2 step (Liston *et al.*, 2001).

Interactions are not only experienced with drugs: constituents of food may also take part in drug interactions. An example of this effect is the well-known inhibitory action of grapefruit juice on some cytochrome P450 isoenzymes, which was discovered rather by chance (Anzenbacher and Anzenbacherová, 2001). In this case, the *in vivo* effect can be quite dramatic with a single glass of grapefruit juice resulting in fivefold increase in the values of the main pharmacokinetic parameters such as the Cmax and Area under pharmacokinetic curve (AUC) for dihydropyridine beta-blocking agents (e.g. nifedipine) (Anzenbacher and Anzenbacherová, 2001).
A group of enzymes located in the endoplasmic reticulum, known as cytochrome P-450 is the most important family of metabolizing enzymes in the liver.

Justification of the work:

A. marmelos is widely used for the treatment of various ailments but little or no interest has been shown in its safety or otherwise. Interestingly, the tree whose leaves and bark are used so widely in treatment of many ailments has seeds which when fresh are edible but are used to make arrow poison to kill animals when dried (Abbiw, 1990). As to whether the other parts, specifically the leaves extract, which are mostly used possesses this toxicity is currently unknown.

Methods and Materials:

Collected leaves were washed under running tap water and kept in shade for drying. Avoidance of any microbial growth on plant material was done by visual observation. Dried plant materials

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were pulverized using mechanical grinder. Pulverized plant materials were observed for colour, odour, and texture, and were packed in air tight container and labeled till any further proceedings.

RESULTS OF PHYTOCHEMICAL SCREENING

The phytochemistry screening of the aqueous freeze-dried extract of ***A. marmelos*** showed the presence for sterols, alkaloids, tannins and reducing sugars. Tests were negative for antraquinones, glycosides, saponins and flavonoids (Table 1).

Table 1: Phytochemical screening of the aqueous extract of *A. marmelos.*

Sterols	Present
Anthraquinones	Absent
Glycosides	Absent
Alkaloids	Present
Saponins	Absent
Flavonoids	Absent
Tannins	Present
Reducing Sugars	Present

DISCUSSION

The preliminary phytochemical screening showed that the aqueous extract of ***Aegle marmelos*** contained tannins, reducing sugars, sterols and alkaloids. This is in confirmation to work done by Ngouela (1988) who found the stem bark to contain sterols as well as triterpenes. The presence of many biologically active phytochemicals such as triterpenes, flavonoids, alkaloids, steroids, tannins and glycosides in various plant extracts may be responsible for their respective pharmacological properties (Agarwal and Rangari, 2003; Liu *et al.*, 1996; Mbagwu *et al.*, 2007; Narendhirakannan *et al.*, 2007; Singh *et al.*, 2002). Alkaloids, which are nitrogen-containing low molecular weight substances, are a major class of plant secondary metabolites that show a wide variety of chemical structures and biological activities. Some are used in both

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modern and traditional medicine. For instance, vincristine and taxol are widely used as anticancer drugs and morphine (in some countries) is an indispensable analgesic in clinical medicine (Otani *et al.*, 2005). Alkaloidal compounds isolated from different parts of several medicinal plants have been reported to be responsible for some pharmacological properties (Duwiejua *et al.*, 2002; Whitehouse *et al.*, 1994). Sterols, specifically phytosterols, more commonly known as plant sterols, have been shown in clinical trials to block cholesterol absorption sites in the human intestine, thus helping to reduce cholesterol in humans (Ostlund *et al.*, 2003). Reducing sugars, with aldehyde or ketone group, in solution is able to act as a reducing agent. Presence or absence of specific phytochemicals in a plant can explain the benefits or dangers the plant contributes when ingested. The presence of sterols, reducing sugars, tannins and alkaloids may confer several pharmacological activities of the plant which may explain the effect of the aqueous leaves extract observed in traditional medicine.

CONCLUSION

In conclusion, preliminary phytochemical screening of the aqueous extract of ***Aegle marmelos*** showed that it contains tannins, reducing sugars, sterols and alkaloids. In this study, the extract exhibited protective effects by interfering with CCl_4 and paracetamol–mediated oxidative stress through decreased bioactivation and production of free radical derivatives evidenced by the decreased cytochrome p450 activity and increased glutathione peroxide and superoxide dismutase activity. ***Aegle marmelos*** exhibits its hepatoprotective potential by modulating activity levels of enzymes and metabolites governing liver function and by helping in maintaining cellular integrity of hepatocytes. Against CCl_4 and paracetamol-induced hepatotoxicity, ***Aegle marmelos*** interferes with generation and reactions of primary radicals (CCl_3/CClOO and NAPQI) and mops up free radicals derived from oxidative stress cause by the primary radicals.

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