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Accepted on: 27.05.2018**Keywords:**Acid neutralizing capacity,
Hydroalcoholic extract,
Madhuca longifolia,
Proton pump inhibition**ABSTRACT**

An ulcer is a common, chronic problem whose incidence is attributed to several factors like stress, food, pathogens and use of drugs which irritate the gastrointestinal tract. The most modest approach in the treatment of ulcer is the inhibition of H⁺K⁺ATPase, the proton pump which functions in the secretion of acid in the gastric cells. The synthetic proton pump inhibitors prescribed to control acid secretion have side-effects affecting the bones and other internal organs. Hence, safe, natural anti-ulcer agents are being researched and identified. The present study investigated the proton pump inhibitory potential of the different concentrations of extracts of the leaves of *Madhuca longifolia*. The extracts exhibited its maximum activity at 80 mg and the potential of the hydroalcoholic extract was comparable to that of the standard drug omeprazole. The acid neutralizing capacity of the extracts was also studied and the capacity was equal to that of the commonly used over-the-counter drug Digene. The results of this study proved that the hydroalcoholic extract of the leaves of *M. longifolia* could be a promising gastro-protective agent.

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INTRODUCTION

Peptic ulceration is a common, chronic, recurrent, and occasionally life-threatening disease in humans. The aetiological factors responsible for ulcer are multiple. The exposure of gastric mucosa continuously to potent injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and drugs results in the ulcer. The pathogenesis of ulcer involves an increase in gastric acid secretion, inhibition of mucosal growth and cell proliferation, and alteration of gastric motility (Selvamathy *et al.*, 2010, Mohammad Shoaib *et al.*, 2016).

Hyperactivity of H⁺K⁺ATPase has been claimed as the primary factor for the excess acidity and mucosal inflammation in gastric ulcer. Hence agents which suppress the activity of H⁺K⁺ATPase activity are considered as a choice of antiulcer drugs.

Active plant metabolites such as flavonoids, phenols and tannins are phytochemicals that can protect against gastric lesions by acting as protective factors or antioxidants. The phytochemicals can inhibit the proton pump of the gastric cells and also assist in neutralizing the excess acid secreted. Herbal medicines are now widely used globally for the treatment or prevention of digestive disorders (Saranya *et al.*, 2011). In traditional medicine, numerous plants and herbs are used to treat gastrointestinal disorders and there has been renewed interest in identifying new gastroprotective agent from natural sources (Sahoo *et al.*, 2016; Abebaw *et al.*, 2017).

Madhuca longifolia Syn. is an economic plant growing throughout the subtropical region of the Indo-Pak subcontinent. Several studies show the presence of secondary metabolites like saponins, triterpenoids, saponins, flavonoids and glycosides in the aerial parts of *M. longifolia*. Traditionally, it

has been used to treat infections, rheumatism, heart disease, diabetes and many other disorders (Sharma *et al.*, 2010, Devi *et al.*, 2015). Most of the scientific reports which establish the gastroprotective effect of *M. longifolia* flowers and seeds attribute the protective effect to the antioxidant potential of the leaves (Seshagiri *et al.*, 2007). The present study aims to ascertain the role of the extracts of *Madhuca longifolia* leaves in inhibiting gastric proton pump.

MATERIALS AND METHODS

Collection of plant material

The leaf part of *Madhuca longifolia* was collected from local suburban areas of Kancheepuram district, Tamil Nadu, India during January 2017. The plant was taxonomically identified by Dr. S. Aravind, Associate Professor, Department of Botany, National Institute of Siddha, Tambaram, Chennai. (Voucher specimen -NISMB2212017)

Preparation of plant extracts

Shade-dried and coarsely powdered leaf part of *Madhuca longifolia* (1kg) was subjected to successive extraction in solvents of increasing polarity (n-Hexane, Chloroform, Ethyl acetate, Ethanol) by using cold maceration technique for 72, 48 and followed by 24 hours. Simultaneously, 1 kg of the dried leaf powder was subjected to exhaustive cold maceration in 70% ethanol for the same duration; Solvents were filtered, distilled and dried in a vacuum desiccator to obtain the extracts.

Study on proton pump inhibition

Preparation of gastric microsomes

The source for H⁺K⁺ATPase was the mucosal scrapings of sheep stomach obtained from a slaughterhouse. The scrapings were homogenized in 200 mM Tris-HCl buffer (pH 7.4) and centrifuged for 10 min at 5000 g. The supernatant was again centrifuged at 5000 g for 20 min to remove fine residues. The protein concentration in the supernatant was determined with bovine serum albumin as a standard. This supernatant was then employed to determine H⁺K⁺ ATPase activity.

Determination of H⁺K⁺ ATPase activity

The H⁺K⁺ATPase activity in the presence of different concentrations of the extracts and omeprazole was assayed by the method of Reyes-Chilpa *et al.*, 2006. The enzyme source was preincubated with different concentrations of the extract (10-80 µg) for 30 min. The assay was conducted in a mixture containing aliquots of the preincubated enzyme, 20 mM Tris-HCl (pH 7.4), 2 mM magnesium chloride and 2 mM potassium chloride. The reaction was started with the addition of 2 mM adenosine-5'-triphosphate (ATP) and incubated for 30 min at

30 °C and terminated by the addition of 10% trichloroacetic acid followed by centrifugation at 2000 g. The amount of inorganic phosphorous released from ATP was determined by the method of Fiske and Subbarow, 1925. The enzyme source was also treated similarly with the standard drug omeprazole and the enzyme activity was measured.

In vitro acid neutralizing activity of the extract

Fifty milligrams of the extract was transferred to 250 ml beaker and 70 ml of distilled water was added. It was mixed with a magnetic stirrer for 1 min. Then 30 ml 1.0 N HCl was added to the test solutions with continuous stirring for 15 min. Excess HCl was titrated with 0.5 N NaOH to attain a stable pH of 3.5. The number of mEq of acid consumed was calculated. The results were expressed as total mEq per gm of substance (USP).

$$\text{MEq} = (\text{V HCl} \times \text{N HCl}) - (\text{V NaOH} \times \text{N NaOH})$$

Where;

V HCl = Volume of HCl added to sample

N HCl = Normality of HCl

V NaOH = Volume of NaOH added to sample

N NaOH = Normality of NaOH

RESULTS AND DISCUSSION

A peptic ulcer is a common lifestyle associated problem faced by the humans of all ages across all regions of the globe. The occurrence of gastric ulcer can be attributed to several factors like H. pylori, stress, junk food, sedentary lifestyle and the use of non-steroidal anti-inflammatory drugs. Gastric ulcer predisposes to gastric cancer and other complications if not treated promptly. A correlation between gastric ulcer and gastric cancer certainly exists. The risk of gastric cancer increases with the duration and severity of gastric ulcer. Hence it is of utmost importance to treat gastric ulcer during its early years of onset.

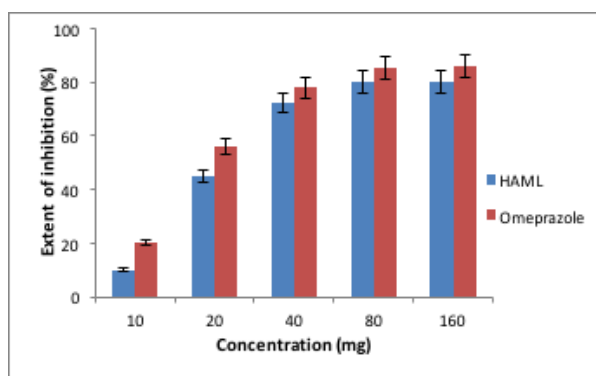


Figure 1: *In vitro* proton pump inhibiting the efficacy of the hydroalcoholic extract of *Madhuca longifolia*

HAML-Hydroalcoholic extract of *Madhuca longifolia*; All values are expressed as Mean \pm S.D (n = 6).

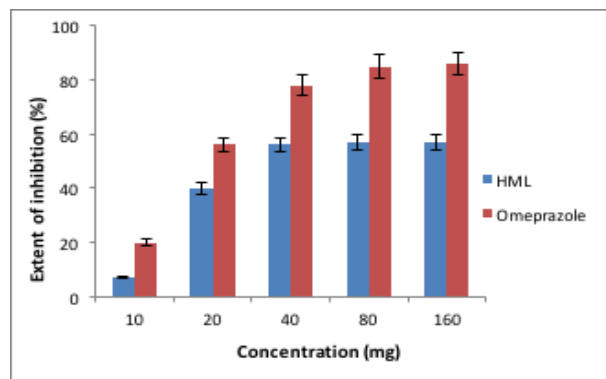


Figure 2: *In vitro* proton pump inhibiting the efficacy of hexane extract of *M. longifolia*

HML- Hexane extract of *Madhuca longifolia*; All values are expressed as Mean \pm S.D (n = 6)

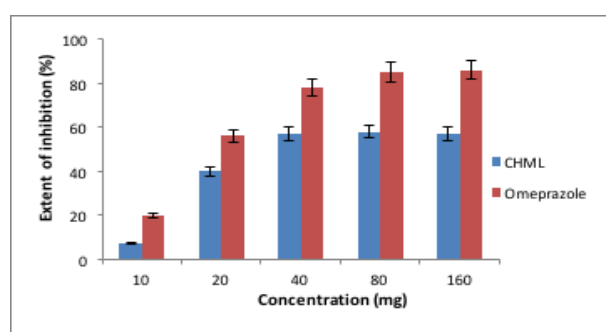


Figure 3: *In vitro* proton pump inhibiting the efficacy of chloroform extract of *M. longifolia*

CHML- Chloroform extract of *Madhuca longifolia*; All values are expressed as Mean \pm S.D (n = 6)

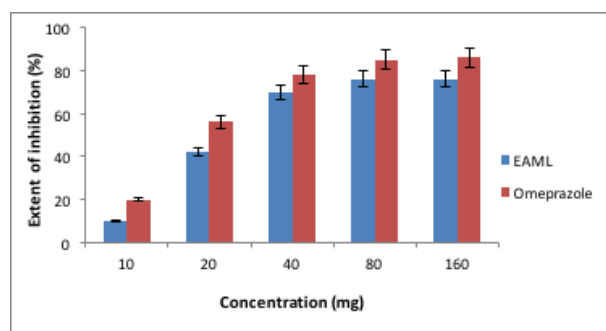


Figure 4: *In vitro* proton pump inhibiting the efficacy of ethyl acetate extract of *M. longifolia*

EAML- Ethyl acetate extract of *Madhuca longifolia*; All values are expressed as Mean \pm S.D (n = 6)

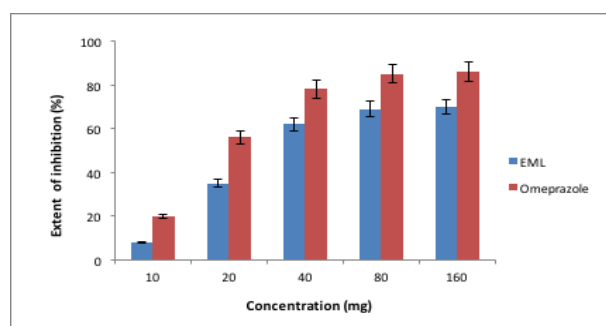


Figure 5: *In vitro* proton pump inhibiting the efficacy of an ethanolic extract of *M. longifolia*

EML- Ethanolic extract of *Madhuca longifolia*; All values are expressed as Mean \pm S.D (n = 6)

Table 1. Acid Neutralizing Capacity of the extracts of *Madhuca longifolia*

Extract	Acid Neutralizing Capacity (mEq)
Hydroalcoholic extract	27 \pm 0.5
Ethyl acetate extract	22 \pm 0.5
Ethanolic extract	15 \pm 0.8
Chloroform extract	14 \pm 0.8
Hexane extract	8 \pm 0.6
Control (100 mg)	
Digene	29 \pm 0.6

The initial step in the onset of gastric ulcer is the mucosal damage which is promoted by the hypersecretion of HCl in the parietal cells. The gastric H⁺K⁺ ATPase (proton pump) is responsible for the acid secretion in the stomach. The major approach in the development of drugs against these acidity related problems is the inhibition of the gastric H⁺K⁺ATPase activity. (Saranya *et al.*, 2011)

Although several proton pump inhibitors such as omeprazole, lansoprazole, ranitidine, famotidine and histamine H₂ receptor blockers are being used to control acid secretion, they produce adverse side effects on human health. The introduction of proton-pump inhibitors for anti-ulcer therapy had revolutionized the treatment of peptic ulcers. However, there is still no complete cure for this disease. It has been shown that long-term use of these drugs leads to various adverse side effects. Thus, there is a need to identify more effective and safe anti-ulcer agents. Many studies have been launched to identify new anti-ulcer therapies from natural sources. Herbs, medicinal plants and crude drug substances are considered to be a potential source to combat various diseases including gastric ulcer (Menaga *et al.*, 2017).

M. longifolia is traditionally acclaimed for its various medicinal values and various researchers have proved the folklore claims of those medicinal values. The bark and seeds of the plant have been much explored for their medicinal values (Devi *et al.*, 2015). This study tries to establish the gastroprotective potential of the commonly available leaves of *M. longifolia*.

The extracts of the leaves of *Madhuca longifolia* were studied for its efficacy in inhibiting the proton pump of gastric microsomes. The inhibition of the proton pump was studied over a

concentration range of 10 mg to 160 mg (Figures 1-5).

The inhibition was found to be the maximum at 80 mg for all the extracts. No increase in the inhibitory potential was observed when the concentration of the extracts was increased to 160 mg. The percentage of inhibition of proton pump of the hydroalcoholic extract was 80% and the effect was comparable with the standard proton pump inhibitor drug, omeprazole which exhibited 86% inhibition (Figures 1 - 5).

The acid neutralizing capacity (ANC) of 80 mg of extracts was also analyzed to understand the efficacy of the extract in protecting the gastric region. The ANC of the hydroalcoholic extract was comparable to the ANC of the commonly used over-the-counter drug, Digene (Table 1).

Long-term use of synthetic PPIs may be associated with a reduction in bone density, progressive renal damage, hypomagnesemia, dementia etc. (Arj *et al.*, 2016). However, some reports contradict this negative association of synthetic PPIs (Madanick, 2013). However, there is no such report available on the negative association between natural PPIs and general health. Hence the hydroethanolic extract of the leaves of *M.longifolia* can be a promising extract with appreciable proton-pump inhibiting capacity and ANC as its potential was comparable to the standard and commonly used synthetic drugs. This gastroprotective effect of the hydroalcoholic extract of *M.longifolia* needs to be validated in animal models.

CONCLUSION

Gastric ulcers induced by several underlying factors can be prevented and cured by inhibiting acid secretion in the gastric cells and by neutralizing the excess acid secreted. These can be done with the aid of natural alternatives to synthetic proton pump inhibitors and acid neutralizers. The results of the present investigation reveal that the hydroalcoholic extract of the leaves of *Madhuca longifolia* is a potent, natural gastroprotective agent which functions by inhibiting acid secretion in the gastric cells by inhibiting the proton pump and by exhibiting acid neutralizing capacity.

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