To study the Antioxidantactivities, Total Phenolic Compounds and FlavonoidsContentin MANILKARA ZAPOTA

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ABSTRACT:-

The aim of this study was to evaluate antioxidant properties of Manilkara zapota.The results of this studies revealed that methanol extracts of bark , fruit and leaves of Manilkara zapota have been reported to possess antioxidant activities. Ethyl acetate fraction of bark extract (BE) exerted strong antioxidant activities with EC_{50} values of BE were 1.42, 4.83 and 53.2 µg/ml in DPPH, $ABTS^{\cdot}$ $^{+}$, superoxide radicals scavenging methods, respectively. The bark of Manilkara zapota may be utilized as effective and safe antioxidant source searching for further bioactive compounds.

Keywords-Manilkara zapota, DPPHradicalscavenging, $ABTS^{+}$, antibacterial activity.

INTRODUCTION:-

Medicinal Plants represents one of the most important fields of traditional medicine all over the world. The study of plants requires the effective integration ofinformation on chemical composition of extracts, pharmacological activities of isolated compounds, as well as indigenous knowledge of traditionalhealers[1]. Despite the discovery of natural products from higher plants, the interest of chemists, Pharmaceutical scientists and Pharmacologists turned to the production of synthetic compounds. In the late 19th century, research was focused mainly on the modification of natural products, to enhance biological activity to increase selectivity and to decrease toxicity and side effects. In more recent years, industry has showed more interest in field of natural product research [2].

Plants are rich in a variety of natural compounds that influence antioxidant and antimicrobial properties have been used for medicinal and food preservative purposes [3]. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man.[4]The effect of plant extracts on bacteria have been studied by a large number of researchers in different parts of the world.[5,6] Manilkara zapota (Family: Sapotaceae) has traditionally been used for its medicinal activity. Several studies showed that different parts of Manilkara zapota such as leaves, fruit, seed, bark and flower possess antimicrobial activity. The leaves of plant contain a bitter principle alkaloid that comprise a stable antioxidant activity. The flower has been used for traditional medicine to relieves as well as prevents the respiratory disorders , fever and pulmonary complaints.[7] The leaf of plant is used to cure common cold, diarrhea , fever wound andulcer etc[8]. Fruits are edible , sweet with rich fine flavor. Fruit of Manilkara zapota has also been used as anti-diarrheal , hemorrhoid aid. Bark is used in the treatment ofdiarrhea , peludism and dysentery9.

Materials andMethods-

Collection and Identification of Plant:- The bark, fruits and leaves of M. zapota were collected from Local area of Doraha , District Ludhiana, Punjab. A specimen copy was deposited to Department of Chemistry, Desh Bhagat University, Mandi Gobindgarh for future.

Preparation of the MethanolExtract

The dried (60℃, 48 h) and finely ground samples of bark, leaves and fruits (6 g each) were separately extracted with 35 ml of 100% methanol for 12 h at room temperature with shaking. After filtration, the plant materials were extracted twice in the same conditions. The methanol extracts obtained from each sample were collected, filtered, dried under vacuum and then re-dissolved in methanol and stored under refrigeration for further analysis.

DPPH radical scavengingassay

Free radical scavenging activity of extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)method[10]. The measurement was performed using a UV-Vis spectrophotometer.

Total antioxidant activity (ABTSassay)

The total antioxidant activity values of M. zapotaextracts were measured by the improved $ABTS^{\cdot+}$ method method as described by Baltrusaityte (2007) [11]with minor modification

PMS-NADH system superoxide-radical scavengingassay

The superoxide scavenging ability of extracts was assayed by the method of Lau et al. (2002)[12] with minor modifications.

RESULT-. The bark extract possessed higher antioxidant activity than other parts, it was further separated into fraction BH, BE and BW. Fraction BE was the most effective in scavenging DPPH and $ABTS^{\cdot}$+ free radicals, scavenging superoxide radicals generated in PMS-NADH system as shown in **(Table-1).**As showed in **(Table- 2)**, catechol exhibited a very strong radical scavenging activity (EC_{50}: 1.28 and 114 µg/ml for DPPH and PMS-NADH assays, respectively), whichwas higher than that displayed by Trolox. Likewise, p-vinylguaiacol and syringol effectively scavenged DPPH free radicals (EC_{50}: 8.76 and 5.44 µg/ml, respectively.

Table (1): Antioxidant effect (EC_{50}) of M.zapota extracts in DPPH radical scavenging, $ABTS^{+}$ radical scavenging and PMS-NADH superoxide radical scavenging assays.

EXTRACTS	EC50 (µg/ml)		
	$ABTS^{+}$assay	DPPHassay	PMS-NADH assay
Bark	$3.6 \pm 0.0_d$	$6.9 \pm 0.1_d$	$87.5 \pm 2.8_d$
Fruits	$8.2 \pm 0.1_c$	$6.3 \pm 0.0_d$	$169 \pm 2.8_c$
Leaves	$9.2 \pm 0.0_b$	$20.4 \pm 0.1_b$	$213 \pm 2.2_b$
BH	$255 \pm 10.1_a$	$369 \pm 13.0_a$	$>1000_a$
BE	$1.4 \pm 0.0_e$	$4.2 \pm 0.0_e$	$53.2 \pm 1.3_f$
BW	$3.8 \pm 0.0_d$	$10.6 \pm 0.1_c$	$73.9 \pm 2.8_e$
Trolox	$0.5 \pm 0.0_f$	$4.4 \pm 0.0_e$	$181 \pm 5.8_c$

Concentration of samples in assays was expressed as final concentration; superscript letters with different letters in the same column indicate significant difference (P<0.05). Each value in the table was expressed as mean ± SD (n=3). (BH,BE and BW were hexane, ethyl acetate and water fractions of bark extract of M.zapota, respectively)

(Table -2): Antioxidant effect (EC_{50}) of standard phenolic compounds in DPPH radical scavenging, and PMS-NADH superoxide radical scavenging assays

Samples	DPPH assay	PMS-NADH assay
Catechol	1.3 ± 0.0	114 ± 7.9
Vanillin	>1000	425 ± 23.3
Syringaldehyde	74.1 ± 0.7	274 ± 26.9
p-Propylphenol	320 ± 3.0	>1000
p-Vinylguaiacol	8.8 ± 0.0	>1000
Syringol	5.4 ± 0.0	>1000

Each value in the table was expressed as mean ± SD (n=3).

Total Phenolic Compounds and FlavonoidsContent-

As one of the most important antioxidant plant components, phenolic compounds are widely investigated in many medicinal plant and vegetables. The amounts of total phenolic compounds and total flavonoids in the methanol extracts of bark, fruits and leaves as well as BH, BE and BW fractions prepared from the bark of M. zapotaare shown in (**Table -3).** The bark contained a significantly higher amount of total phenolic than fruits and leaves (237, 179 and 127 mg/g extract, respectively). Furthermore, total phenolics were accounted in greater amounts in BE than BW and BH fractions prepared from the bark and were 436, 194 and 41.7mg GAE/g extract,respectively

Samples	Total phenolic content as gallic acid equivalents (GAE mg/g extract)	Total flavonoid content as rutin equivalents (GAE mg/g extract)
Bark	$227 \pm 0.6_b$	5.3 ± 0.03
Fruits	$169 \pm 6.8_d$	$5.1 \pm 0.03_c$
Leaves	$118 \pm 1.2_e$	$8.4 \pm 0.6_a$
BH	$40.7 \pm 1.0_f$	$1.2 \pm 0.1_e$
BE	$416 \pm 2.1_a$	$6.0 \pm 0.1_b$
BW	$184 \pm 0.8_c$	$1.6 \pm 0.1_d$

Each value in the table is represented as mean ± SD (n=3); superscript letters with different letters in the same column indicate significant difference

(P<0.05); BH, BE and BW are hexane, ethyl acetate and water fraction of M. zapotabark extract respectively

DISCUSSION-

The results in this study revealed that the methanol extracts from bark, fruits and leaves of M.zapota showed strong antioxidant and antibacterial effects and contained high amounts of phenolic compounds. Ethyl acetate fraction of bark extract (BE) exerted strong antioxidant effects and EC_{50} values of BE were 4.2, 1.4 and 53.2 µg/ml in DPPH, $ABTS^{\cdot}$, $\overset{\cdot}{s}$uperoxide radicals scavenging methods, respectively. Totalphenolic were accounted in greater amounts in Bark extract.

CONCLUSION: -Results showed that methanol extracts of bark, fruits and leaves of M. zapota exhibited excellent antioxidant activities and high level of phenolic compounds.

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