Phytochemical and Biological Study on *Schotia brachypetalae* Family Leguminosae (Fabaceae)

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To My Father “Dr Ashraf ”, My Mother “Dr Nagwa” and My lovely Husband "Eng. Mohamed"

To the soul of my Grandmother and my Father-in-law

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$ABTS^{\cdot+}$	[2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]
CC	column chromatography
cm	centimeter
CMC-Na	carboxymethyl cellulose sodium
2D	two dimensional
Da	dalton
DCF	2', 7'- dichlorofluorescein
DCFDA	2', 7'- dichlorofluorescin diacetate
DMSO	dimethyl sulfoxide
Dil	diluted
$DPPH^{\cdot}$	(2, 2-diphenyl-1-picrylhydrazyl)
DW	dry weight
ELISA	enzyme-linked immunosorbent assay
$ESI/LC/MS^n$	electrospray ionization- liquid chromatography-mass–mass spectrometry
g	gram
GFP	green fluorescence protein
^{1}H-NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography
hsp	heat shock protein
Hz	hertz
J value	coupling constant
Kg	kilogram
L	liter
m/z	mass to charge ratio
mg	milligram
MPO	myeloperoxidase
NSAID	non-steroidal anti-inflammatory drugs
PC	paper chromatography
PGE_2	prostaglandin E_2
PPC	preparative paper chromatography
ppm	part per million
R^2	regression coefficient
RBCs	red blood cells
ROS	reactive oxygen species
RSD	relative standard deviation
R_t	retention time
S.D.	standard deviation
TNF-α	tumor necrosis factor α
UV	ultraviolet
δ	chemical shift
λ	wave length
µl	microliter

Introduction

Introduction

During the last few decades, there has been an increasingly intensified search for biologically active compounds of natural origin. Over this period, a worldwide interest in drugs derived from plants has been developed to produce a daily increase of public demand of these drugs for treatment of broad range of diseases. Many of these drugs possess multiple biological effects as anticancer, antiviral, antihyperglycemic or immuno-modulating agents (Dahanukar *et al.* 2000).

Phenolic compounds are found in both edible and non-edible plants and they have been reported to have a lot of biological effects. Crude plant extracts rich in phenolics are of increasing interest for biological and phytochemical investigations. Flavonoids and other phenolics have been suggested to play a preventive role in cancer development and heart diseases (Dahanukar *et al.* 2000).

Among the famous plant families which include genera that embraces phenolic-rich species, Leguminosae (Fabaceae) occupies a distinguishable situation. This is due to the fact that many of its plants are capable of synthesizing and accumulating high percentage of phenolics. Fabaceae is a large family, distributed in tropical and subtropical regions. The family is considered the second largest family among the flowering plants, behind Asteraceae, with 730 genera and over 19,400 species. It is a large family of trees, shrubs and herbs bearing bean pods distributed throughout the world.

Leguminosae is one of the most important families from an economic point of view. The seeds are rich in starch and protein, used as widespread source of food as in various beans (Rendle 1959).

Legumes, broadly defined by their unusual flower structure, podded fruit, and the ability of 88% of the species examined to date to form nodules with rhizobia. In addition to traditional food and forage uses, legumes can be milled into flour, used to make bread, doughnuts, tortillas, chips, spreads, and extruded snacks or used in liquid form to produce milks, yogurt, and infant formula. Pop beans, licorice (*Glycyrrhiza glabra*), and soybean candy provide novel uses for specific legumes. Legumes have been used industrially to prepare biodegradable plastics, oils, gums, dyes, and inks. Galactomannan gums derived from *Cyamopsis* spp. and *Sesbania* spp. are used in sizing textiles and paper, as a thickener, and in pill formulation (Graham and Vance 2003).

Many legumes have been used in folk medicine. Isoflavones from soybeans and other legumes have more recently been suggested both to reduce the risks of cancer and to lower serum cholesterol. Soybean and soyfood phytoestrogens have been suggested as possible alternatives to hormone replacement therapy for postmenopausal women (Graham and Vance 2003).

Schotia brachypetala Sond. tree is indigenous to South Africa (Watt and Breyer-Brandwijk 1932, Brenan 1967). *Schotia* was named after Richard Schott and *brachypetala* means having short petals in Greek and refers to the flowers which are unique among *Schotia* species in that the petals are partially or completely reduced to linear filaments. The beauty of the flower is in brightly coloured sepals, stamens and pedicels. The flower produces copious amounts of nectar which overflows and drips or weeps (Germishuizen *et al* 2006).

Traditionally, the bark decoction was used to strengthen the body, treat dysentery and diarrhea, nervous and heart conditions, flu symptoms and an emetic in excessive beer drinking, the bark is also used for dyeing giving reddish brown or red colour. Its roots were also used to treat diarrhea and heartburn. The seeds are edible after roasting (Du *et al.* 2014). The timber is of good quality suitable for furniture making and flooring blocks and said to be excellent for all kinds of wagon wood (Germishuizen *et al* 2006).

Aim of Work

Aim of Work

To the best of our knowledge, there was little information concerning *Schotia brachypetala* Sond., cultivated in Egypt. It was therefore, found interesting to subject the extract of the leaves of entitled plant to an intensive biological and phytochemical investigations.

Pilot study on the methanolic leaf extract of *Schotia brachypetala* Sond. using 2D paper chromatography showed a complicated mixture of polyphenolics, this directed the present study to investigate in-depth the phenolics of the leaf extract. Besides evaluation of the biological activity of the extract by determining its anti-inflammatory and anti-oxidant activities. Our work strategy included:

1. Phytochemical investigation of the methanolic leaf extract of *Schotia brachypetala* Sond. The study included: phytochemical screening and phytochemical investigation of the methanolic leaf extract in an effort to fractionate and isolate phenolic compounds. Identification of the isolated compounds using spectroscopic methods e.g. UV, ^{1}H-NMR and LC/ESI/MS^{n} spectrometry. Also, it will enable the correlation between the biological activity and the chemical constituents whenever possible. Together with determination of the total phenolic and flavonoidal content of the methanolic leaf extract of of *Schotia brachypetala* Sond.
2. Biological investigation of the methanolic leaf extract of *Schotia brachypetala* Sond. It included: screening for the anti-inflammatory activity in two experimental models, also screening for the anti-oxidant activity in different *in-vitro* and *in-vivo* models.
3. Standardization of the methanolic leaf extract of *Schotia brachypetala* Sond. using HPLC analysis.