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Evaluation of Phytochemical Screening and Antifungal Activity for Some Annual Plant Extracts in Egypt

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ABSTRACT

Egypt has many wild plants such as *Sonchus oleraceus*, *Cichorium pumilum* and *Portulacaoleraceae which* are one of the perfect sources of natural effective compounds including alkaloids, flavonoids, tannins, terpenes, steroids and phenols. This study investigated that *P.oleracea* has a greater total phenolic, flavonoids and antioxidant activity followed by *S. oleraceus* and finally *C. pumilum.* Also, alkaloid was found in all the plants examined with the highest quantities obtained in *S. oleraceus* (78.6%) then, *P. oleracea* (66.4%), while *C. pumilum* have 41.8%. While total terpenoid and saponin were the highest percentages with *Portulaca oleracea* (61.6%) and (35.3%) respectively. In addition to all the extracts could inhibit these fungi at a different level in which ethanolic and methanolic extracts of Portulaca oleracea and Cichorium *pumilum* generally had higher antifungal activities against *A. aflatoxiformans* and *A ochraceus.* Finally, these plant extracts contain many effective compounds that enable to play the role of antifungals.

INTRODUCTION

Many wild plants grow in Egypt used in treating certain diseases. Three wild edible plants (Sonchus oleraceus) known as Sow Thistle and (Cichorium pumilum) belonging to the Asteraceae family. Portulaca oleracea is an annual succulent in the family Portulacaceae (Abou El Seoud *et al.*,2003; Petropoulos *et al.*,2019; Almashad *et al*. (2019,). *Sonchus oleraceus* (L.) is a plant that belongs to the family asteraceae which is used in medicine for the treatment of gastrointestinal tract disorder in addition it is used as food in some parts of Africa and Asia (Hussain *et al*.,2010). The genus Sonchus comprises about 60 species, three of which have become a common weed around the world. These are *S. arvensis*, perennial Saw thistle and the two annual species *S. oleraceus*, and *S. asper*.

S. oleraceus is a common winter weed in Egypt found in various locations, such as orchards, crop fields, gardens, canal banks, roadsides, fallow lands and even at sites of uncontrolled water runoff. (Gomaa *et al.*, 2012).

Many compounds identified in the leaves of *S. oleraceus*, including alkaloids, tannins, terpenes, flavonoids, steroids and phenols (Yin *et al*. 2007; Singh 2010). The genus *Sonchus* is characterized by the presence of sterols, triterpenes and their glycosides (Jain and Singh.,2014). Delyan (2016) reported the presence of 26 compounds in leaves of *Sonchus Arvensis L.* using GC-MS analysis, some of the phytocomponents screened were phytol, pentacosane, 1, 2-benzenedicarboxylic acid, bis (2-methylpropyl) ester. The genus Cichorium comprises seven species native to the Mediterranean basin (Pinelli *et al*.,2007). It is known as an undesired weed infecting crops in Egypt. Previous studies stated that Cichorium spp. contains several phenolics (Kisiel and Michalska, 2006). Aisa *et al. (*2020) reported that the genus Cichorium are famous due to their therapeutic and medicinal properties. They were several compounds belonging to sesquiterpenoids, steroids, coumarins, flavonoids, triterpenoids and others. Pharmacological effects such as photo-protective, hepatoprotective, anti-diabetic and lipid-lowering, antioxidant, anti-inflammation, antifungal, antimalarial, increased bone mineral density, as well as antitumour activity. Al-Snafi (2016) investigated that the different parts of *Cichorium intybus* contain sesquiterpene lactones (especially lactucin, lactucopicrin, 8-desoxy lactucin, guaianolid glycosides, including chicoroisides, coumarins,sonchuside C), caffeic acid derivatives, inulin, sugars, proteins, hydroxycoumarins, flavonoids, alkaloids, terpenoids, oils, steroids, volatile compounds and vitamins.

Portulaca oleracea L., belonging to the Portulacaceae, it is a warm herbaceous annual plant. It is commonly known as purslane (USA and Australia), rigla (Egypt) and pigweed (England) (Elkhayat *et al*.,2008). Almashad *et al*. (2019) showed that pyrogallol was the main phenolic compound in Egyptian purslane fresh leaves with a concentration of 24.85 and 23.65%, respectively using HPLC analysis. On the other hand, the phenolic compounds in purslane have a high amount of chlorogenic and salicylic followed by, catechin, rosmarinic, vanillic and rutin. Also, a total of 27 compounds were identified using GC-MS in *P. oleracea*. The main volatile compounds were: (E)-2- Hexenal (15.64%), (E)-2-Nonenol (12.03%), Hexanal (10.92%), and Ethyl linoleate (8.02%). Dabbou *et al*. (2020) evaluated six volatile classes with monoterpene hydrocarbons, oxygenated monoterpenes, and non-terpene derivatives as the highly represented compounds. Limonene (17.3–32.2%), carvone (38–46%), 2,6-dimethylcyclohexanol (2.2–6.4%), and nonanal (3.4–3.8%) were the most abundant volatiles in *P. oleracea* using gas chromatography-mass spectrometry (GC-MS).

The objective of this study includes phytochemical screening and quantification of primary and secondary metabolites like chlorophyll, carbohydrates, protein, lipids, phenol, tannin and flavonoids from Sonchus oleraceus, Cichorium pumilum and Portulaca oleracea leaves.

MATERIALS AND METHODS

Plant Materials:

Fresh shoots of *Sonchus oleraceus, Cichorium pumilum* and *Portulaca oleracea* were collected from various cultivated fields in Qalubyia governorate. The leaves were dried by air, then ground to a fine powder and stored in a plastic package.

Fungal Strains:

The fungal strains from A*spergillus* spp. as A.*aflatoxiformans* and *A ochraceus* isolated from cereals.

Preparation of Plant Extracts:

The plant material was air-dried and pulverized into powder, 50 g per 250 ml of each solvent such as methanol, 70% ethanol,

acetone and water extracts were prepared separately and kept in shaking for 24 h. After extraction, the extracts were filtered through filter paper and the filtrate was evaporated to dryness. The concentrated extracts were subjected to qualitative tests for the identification of most phytochemical constituents.

Qualitative Phytochemical Screening of Extracts:

Methanol, 70%ethanol, acetone and water extracts were used for preliminary phytochemical analysis, the following qualitative tests for both metabolites were done as follows:

1-Test for alkaloid:

Extracts treated with a few drops of dil HCl, filtered and subjected filtrate to the number of tests.

Mayer's test: To 3 mL filtrate, a few drops of Mayer's reagent were added. The appearance of cream-colored precipitates revealed the presence of alkaloids.

Hager's test: To 2 mL filtrate, few drops of Hager's reagent were added. The appearance of yellow colour precipitates revealed the presence of alkaloids.

Wagner's test: To 3 mL filtrate a few drops of Wagner's reagent were added. The appearance of reddish-brown precipitates revealed the presence of alkaloids.

2-Test for Terpenoids:

Salkowski test: 2 ml of each extract was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddish-brown color indicated the presence of terpenoid. (Kumar and Patra, 2017).

3-Test for Flavonoids:

Sodium hydroxide test: Few quantities of extract dissolved in water and filtered; to this 2ml of the 10% aqueous sodium hydroxide was later added to produce a yellow coloration. A change in color from yellow to colorless on the addition of dilute HCL indicated the presence of flavonoids. (Kumar and Patra, 2017).

4-Test for Phenolic Compounds:

A few drops of ferric chloride solution were added to the extracts and the formation of bluish-black or dark green color proves the presence of phenols (Tiwari *et al*.,2011).

5-Test for Tannins:

a-Ferric chloride test: 1ml of the extract was boiled in 20ml of water then filtered. A few drops of 0.1% ferric chloride added a green or a blue-black coloration which confirms the presence of tannin. (Ajiboye *et al*.,2013).

b-Alkaline reagent test: Extracts were treated with 10% NaOH solution formation of intense yellow color indicated the presence of tannins. (Kumar and Patra, 2017).

6-Test for Steroids:

Lieberman Burchard test: Chloroform solution of the extract-treated with few drops of acetic anhydride and one ml of concentrated sulphuric acid gives reddish ring at the junction of 2 layers changes into green color indicates the presence of sterols. (Sheel *et al*.,2014).

7-Test for Protein & Amino Acids:

Ninhydrin test: About 0.5 mg of extract was taken and 2 drops of 0.2% ninhydrin reagent were added and heated. The appearance of pink or purple color indicates the presence of proteins or amino acids. (Geetha and Geetha, 2014).

8-Test for Carbohydrates:

Fehling's test: an equal volume of Fehling solution A and B were added to an equal volume of filtrate and boil in a water bath. The formation of a red precipitate indicates the presence of carbohydrates. (Desilva *et al*.,2017).

9-Test for Saponin:

Foam test: 0.5 mg of extract diluted with 20 ml distilled water and shaken or 15 min. The formation of foam to a length of 1cm indicated the presence of saponins and steroids according to the method described by Geetha and Geetha (2014).

Quantitative Secondary Phytochemical Estimation:

Total Phenolic Content (TPC):

The total phenolic content in the extract of leaves was determined using Folin–Ciocalteau method as described by Turkoglu *et al*. (2007).

Total Flavonoid Content (TFC):

The total flavonoid content of different extracts leaves was determined according to the method described by Turkoglu *et al*. (2007) the absorbance was determined spectrophotometrically at 415 nm. The total flavonoid content was measured by plotting the calibration curve of a quercetin standard.

Determination of Antioxidant Activities (DPPH method):

The antioxidant activity of extracts, based on the scavenging activity of the stable DPPH free radical was determined by the method described by Lee *et al.* (2004)

Total Alkaloid Estimation:

5g of each plant parts sample was weighed and added 200ml of 10% solvent of acetic acid in ethanol then make evaporations of solvent and allowed to stand for 4 hours. ammonium hydroxide was added dropwise into concentrated extracts until the precipitation was completed. The solution was allowed to settle the precipitate and filtered. Filtered precipitate washed with dil. ammonium hydroxide and then again filtered. This precipitate residue is an alkaloid that was dried and weighed (Kumar and Patra, 2017).

Total Saponin Estimation:

10g of a plant powder sample in 100ml of 20% ethanol. This sample suspension was heated over a water bath for 4hour at 55ºC with continuous stirring. This sample was filtered and the extract was collected in a 200ml capacity of the beaker. The concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. 30 ml of n-butanol was added. The combined n- butanol extracts were washed with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven and weighted.

Estimation of total Terpenoids:

5g of plant powder soaked in alcohol for 24 hours, filtered. The filtrate was extracted with petroleum ether and the ether extract was treated as total terpenoids. The residue obtained was dried and weighed according to Tejavathi and Jayashree. (2013).

Assay of Antifungal Activity:

The antifungal activity of different plant extracts was tested on *A.aflatoxiformans* and *A ochraceus* using the agar well diffusion method. 1 ml of spore suspension was inoculated into each plate containing 25 ml of sterile PDA medium. Wells of 5 mm diameter were made on the PDA surface and filled with (300µl) of extracts. The plates were incubated at 28 ºC. After incubation time, the plates were tested for the mycelial growth inhibitory zones around the wells.

RESULTS

Phytochemical Screening for Plant Leaves Extracts:

The plants always contain many natural compounds with novel structures and this keeps the investigators interested in researching many plant species till today. The percentage yield of different plant extracts recorded in (Fig. 1). The results revealed that the aqueous extract had the highest yield (15.2%), (14.2%) and (25.4%) with *S. oleraceus*, *C. pumilum*, and *P.oleracea*, respectively. While the acetone extract had the lowest yield.

Data presented in Tables (1,2&3) revealed that the presence of carbohydrate, protein, steroid, Tannins, phenolic, Terpenoid, Flavonoids, alkaloid and saponins as a phytochemical analysis conducted on *S. oleraceus, C. pumilum* and *P. oleracea* extracts. Under the same extraction conditions, the solvent is recognized as one of the most important parameters. According to the extraction yields, the content of most active compounds (phenolics, alkaloids, flavonoids, and terpenoids) varied along with the extracts. the highest levels of phenolics, flavonoids, alkaloids, and terpenoids were observed in aqueous, ethanolic and methanolic extracts.

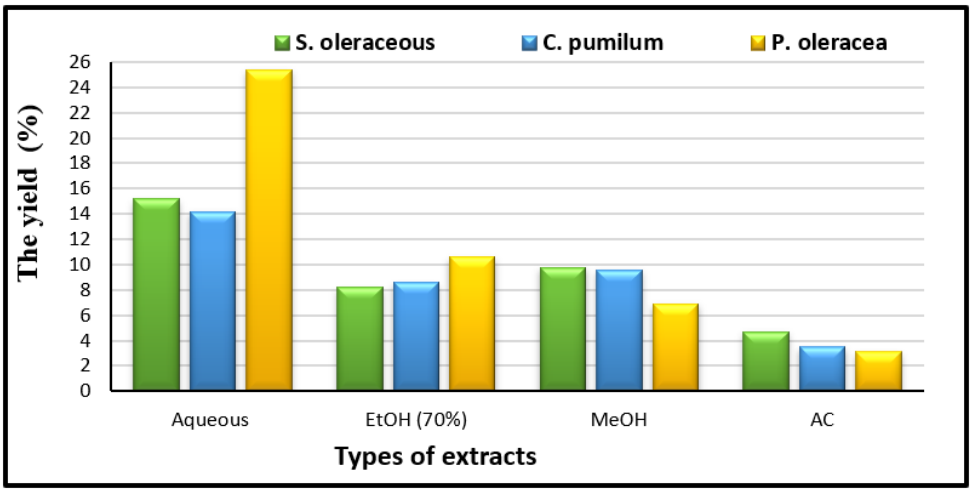


Fig. 1: The percentage of the yield from different plant extracts calculated on the basis of dry weight.

Table 1: Phytochemical screening for leaf extract of *Sonchus oleraceous* with different solvents.

No.	Types of tests		Types of solvents			
			Water	Ethanol (70%)	Methanol	Acetone
1	Terpenoid	Salkowski	+	++	-	-
2	Flavonoids	Sodium hydroxide	+	++	+	-
3	Phenolic content	Ferric chloride test	+	+	-	-
4	Tannins	Alkaline reagent (NaOH)	+	+	+	-
		Ferric chloride test	+	+	-	-
5	Alkaloid	Hager's test	+	+	+	+
		Wagner's test	+	-	+	-
		Mayer's test	+	-	-	-
6	Protein &amino acid	Ninhydrine	+	+	+	+
7	steroids& sterols	Lieberman Burchard test	+	+	+	-
8	Saponins	Foam test	+	+	+	+
9	Carbohydrates	Fehling test	-	+	+	-

+ Present, -Absent.

Table 2: phytochemical screening for leaf extract of *Cichorium pumilum* with different solvents**.**

No.	Types of tests		Types of solvents			
			Water	Ethanolic 70%	Methanolic	Acetone
1	Terpenoid	Salkowski	+++	++	-	-
2	Flavonoids	10%NaOH	-	++	+	+
3	Phenolic content	Ferric chloride test	+	+	-	-
4	Tannins	Alkaline reagent (NaOH)	-	+	+	+
		Ferric chloride test	-	+	-	-
5	Alkaloid	Hager's test	+	+	+	-
		Wagner's test	+	+	+	+
		Mayer's test	+	-	+	-
6	Protein &amino acid	Ninhydrine	+	+	+	+
7	Steroids & sterols	Lieberman Burchard test	-	++	-	+
8	Saponins	Foam test	+	+	+	+
9	Carbohydrates	Fehling test	-	+	+	-

+ Present, -Absent

Table 3: Phytochemical screening for leaf extract of *Portulaca oleracea* with different solvents.

No.	Types of tests		Types of solvents			
			Water	Ethanol (70%)	Methanol	Acetone
1	Terpenoid	Salkowski	+	++	+	-
2	Flavonoids	10%NaOH	-	+	+	-
3	Phenolic content	Ferric chloride test	+	+	-	+
4	Tannins	Alkaline reagent (NaOH)	+	-	-	+
		Ferric chloride test	+	+	-	+
5	Alkaloid	Hager's test	+	+	+	-
		Wagner's test	+	+	+	+
		Mayer's test	+	-	+	-
6	Protein &amino acid	Ninhydrine	+	+	-	+
7	steroids&sterols	Lieberman Burchard test	-	++	+	+
8	Saponins	Foam test	+	+	+	+
9	Carbohydrates	Fehling test	-	+	+	-

+ Present, -Absent

Quantitative Evaluation of Phytochemicals:

1-Total Phenolic Content, Flavonoid Content and Antioxidant Activity:

Data recorded in **Fig. (2,3& 4)** showed that the extract of *P. oleracea* has a greater total phenolic and flavonoids that were 212 & 36mg/mL, respectively, followed by *S. oleraceus* 192& 31 mg/mL and the lowest *C. pumilum* were188 and 27.6 mg/mL. However, DPPH free radical scavenging activity was (57.3%,63.8%&65.6) % with three plants extract *C. pumilum, S. oleraceus* and *P. oleracea*, respectively.

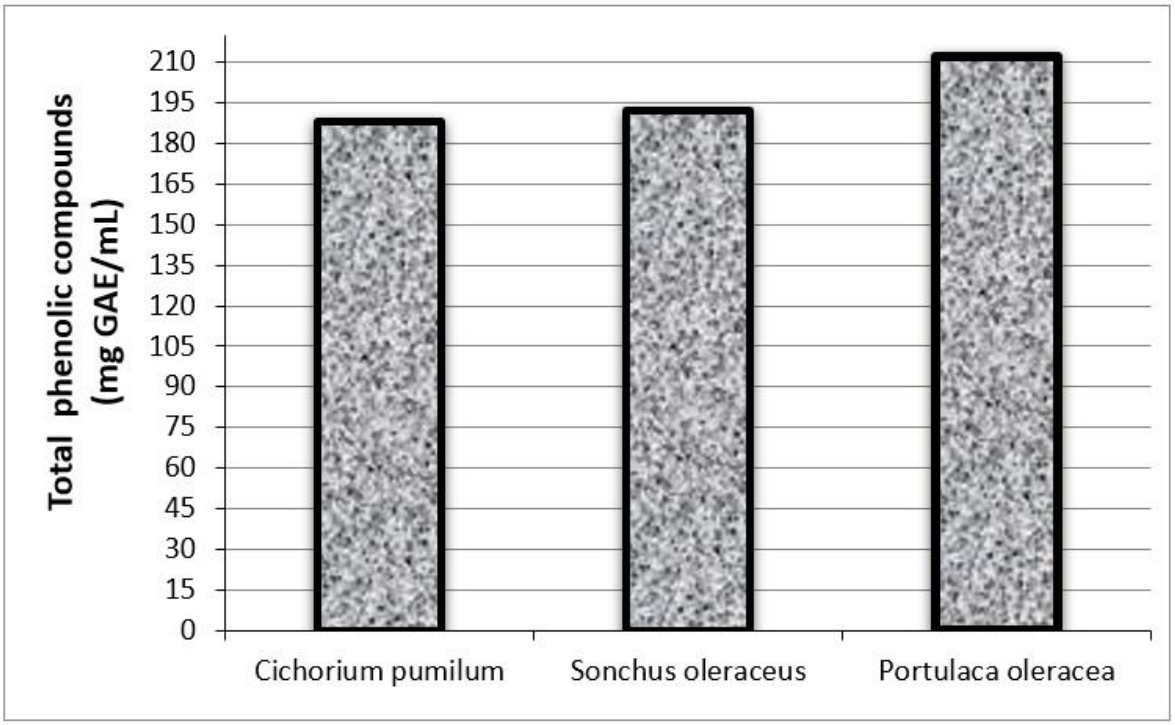


Fig. 2: Total phenolic content compounds as gallic acid equivalent in different plants.

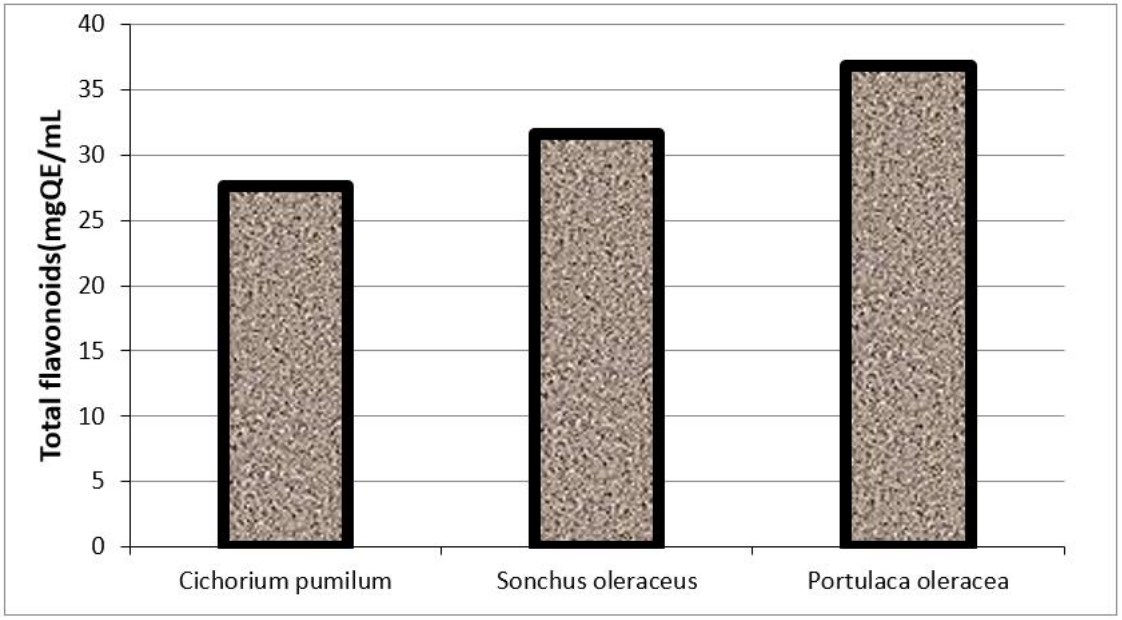


Fig. 3: Total flavonoids content compounds as quercetin equivalent in different plants.

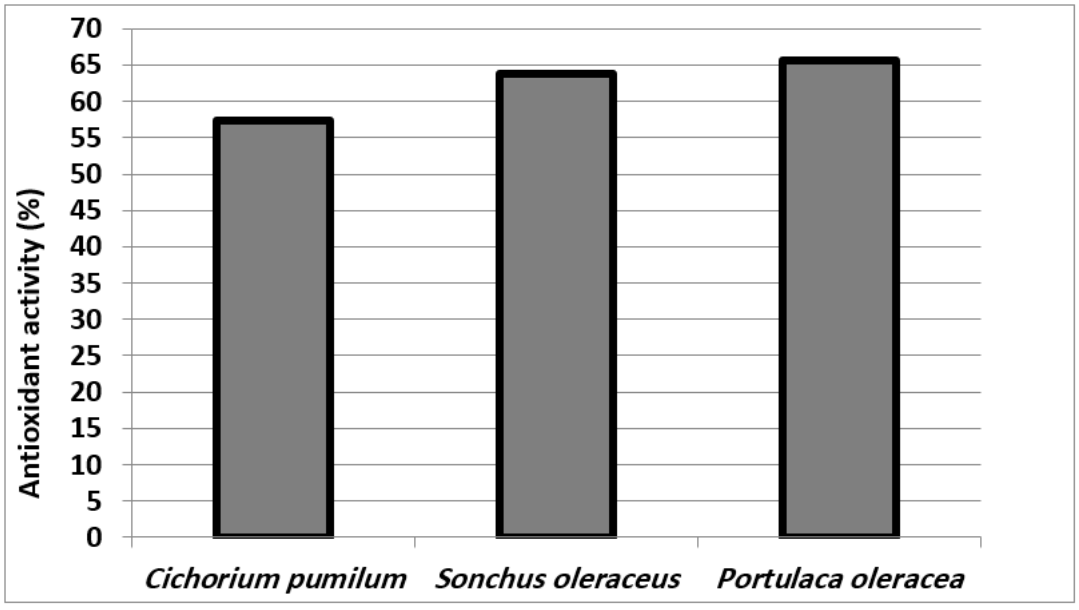


Fig. 4: The percentages of antioxidant activity in different plants.

2-The Quantitative of Total Alkaloid, Terpenoid and Saponin:

The results in **Fig. (5)** indicated that the alkaloid was found in all the plants examined with the highest quantities obtained in *S. oleraceus* (78.6%) than *P. oleracea* (66.4%), while *C.pumilum* have 41.8%. While the total terpenoid and total saponin were the highest percentages with *P.oleracea* (61.6%, 35.3%) followed by *S. oleraceus* were (43.2%,24.8%) and in the case of *C.pumilum* were(23.6%, 25.3%) respectively.

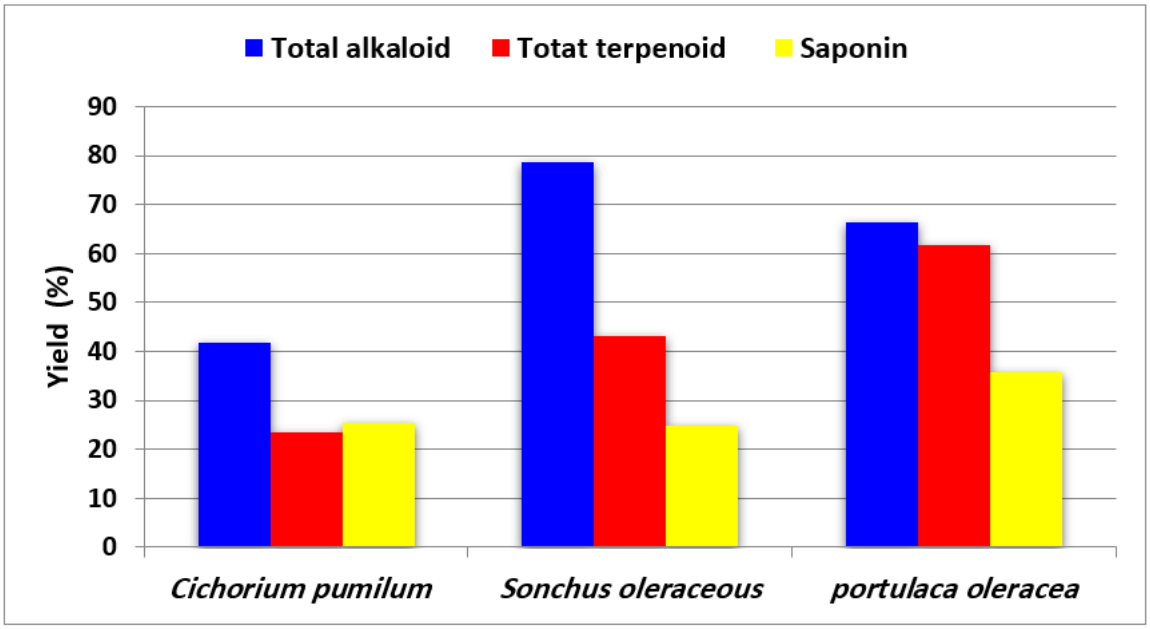


Fig. 5: The percentages of total alkaloid, terpenoid and saponin.

Antifungal Activity of Different Plant Extracts:

The antifungal activity of water, 70%ethanol, methanol and acetone extracts for Sonchus *oleraceus, Cichorium pumilum* and *Portulaca oleracea* were tested for their antifungal effects against *A. flavus* and *A.ochraceous* Fig.(6&7) the results showed that all the extracts could inhibit these fungi at a different level in which ethanolic and methanolic extracts of Portulaca oleracea L and *Cichorium pumilum* generally had higher antifungal activities against *A.aflatoxiformans* and *A ochraceus.* In the case of *A. aflatoxiformans* strain, the highest inhibition zones were (32.3 and 31) mm with methanolic and ethanolic extract of *P.oleracea* While, with *A. ochraceus* the highest inhibition zone were (30 mm) by extract of *C. pumilum* followed by methanolic extract of *S. oleraceus* 27mm Fig.(8).

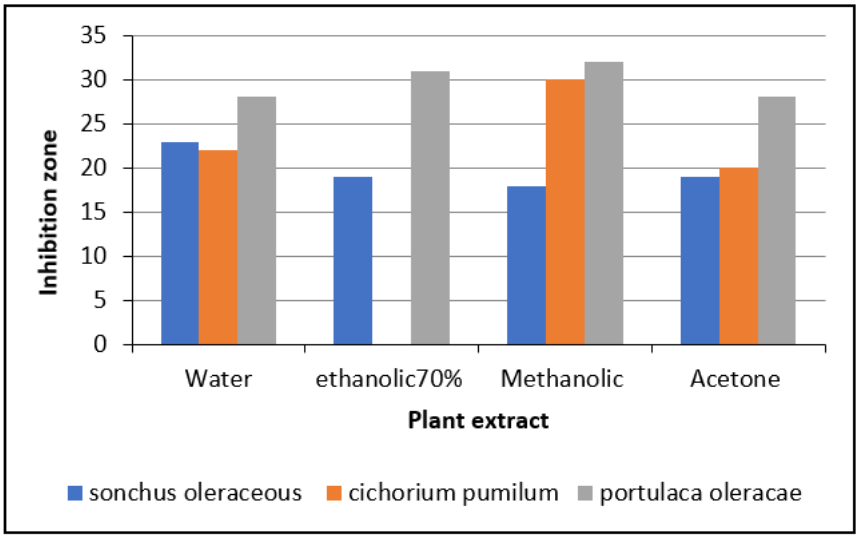


Fig. 6: Inhibition zone (mm) of *Aspergillus aflatoxiformans* after treatment with different plant extracts.

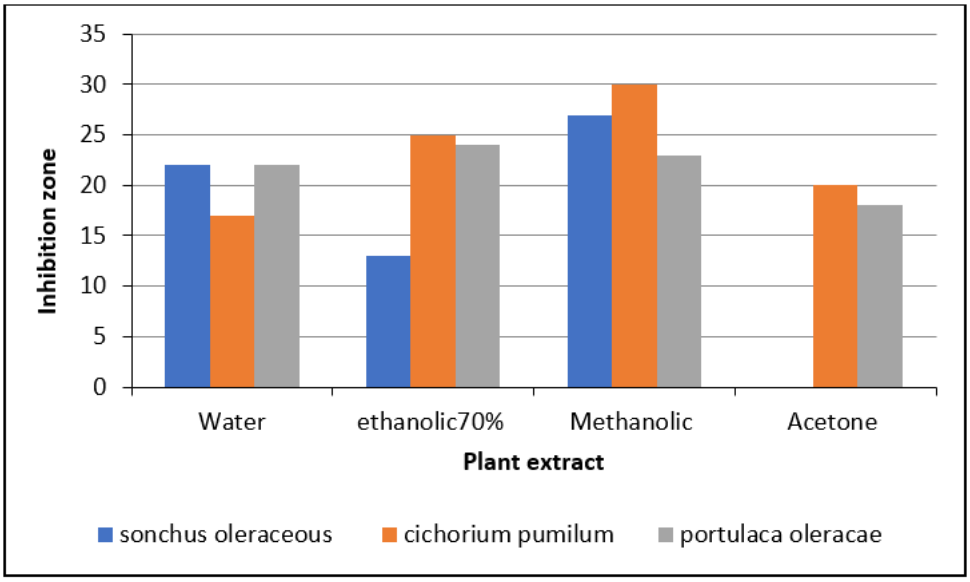


Fig. 7: Inhibition zone (mm) of *Aspergillus ochraceous* after treatment with different plant extract.

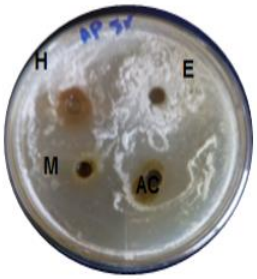
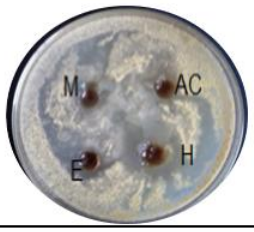
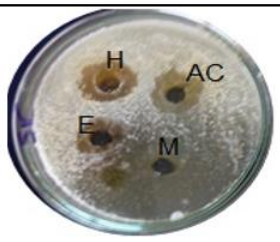
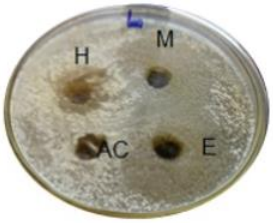
Fungal strain	*portulaca oleracae*	*cichorium pumilum*	*sonchus oleraceous*
A. aflatoxiformans			
A.ochraceous			

Fig.8: Inhibition zone of fungal growth after treatment with plant extracts at concentration (300) µl. H * Water &M* Methanol & E*ethanol 70%& AC*acetone extracts.

DISCUSSION

The extraction yield, phytochemical content and antioxidant properties were influenced by the polarity of extracting solvents (Nawaz *et al*., 2020). The different solvents resulted in various extraction yields this is because differences in the polarity of the solvents could cause a wide variation in the level of bioactive compounds in the extract due to the plant materials containing high levels of polar compounds that are soluble in solvents with high polarity, the solubility of the phenolic compounds affected by the polarity of the solvent used., the determination of phenolic compounds from plants influenced by the extraction, time, the temperature and technique So, it is difficult to develop extraction process suitable for the extraction of all the phenolics in plants (Mahmoudi *et al*., 2013; Brglez Mojzer *et al*., 2016). The different solvent extracts were soluble in different phytoconstituents in different amounts, so, antimicrobial activities were various. saponin, alkaloid, tannin,

flavonoid, terpenoids, protein and carbohydrates have been observed as the active phytoconstituents so it's proved to medicinal implications (Gills ,1992). These indicated the influence of the extraction solvent affected on the total content of phenolic compounds extracted. Solvent types also affect the physical properties of the extracts, especially the solubility of photo components. These results were in agreement with these reports by Okafor and Ezejindu (2014) they found that the presence of photo components including saponin, alkaloid, glycoside, terpenoid, steroid, protein and carbohydrates were accessed qualitatively also they found a low content of flavonoid were 6% and tannin 0.03%in *P. oleracea*. And similar results are given by Abbas *et al*. (2015) which found that the leaves of *Cichorium intybus* possess comparatively higher values of total flavonoids, total phenolic acids, tannins, saponins. The presence of tannin in the plant showed its potential as an antiviral, antibacterial and anti-parasitic (Akiyama *et al*., 2001; Kolodziej and Kiderlen, 2005). Phenolics or polyphenols contain a high amount of antioxidant activities (Razali *et al*., 2008). While flavonoids showed antioxidative, antimicrobial, anti-allergic, anti-inflammatory, anti-diarrhea and anticancer activities (Yamamoto and Gaynor, 2001). The flavonoid content in the leaves was higher than that present in flowers in addition to polar extracts (ethanol and water extracts) showed more flavonoids than non-polar extracts (Rebaya *et al*., 2014). The flavonoids and phenolics were reported to possess antioxidant activities because of the presence of hydroxyl groups in their structures which contribute to a defense system against oxidative injury due to endogenous free radicals (Saggu *et al*., 2014). Phenolic compounds are known to have antioxidant activity and it is likely that the activity of these extracts is due to their content (Okudu *et al.*, 1994; Tepe *et al.*, 2006). In this field, Oliveira *et al*. (2009) showed that the total phenolic of *P. oleracea* stems and leaves was very different between samples, ranging from 78.3 to 633.9 mg/kg dry weight. However, Lim and Quah (2007) reported that the total phenolic of *P. oleracea* ranged from 127 to 478 mg GAE 100 g^{-1}. This may be due to the presence of different amounts of sugars, carotenoids, or ascorbic acid, or other conditions including the duration, geographical variation or extraction methods, growing conditions and cultivation practices which may alter the number of phenolics (Morales *et al*., 2014; Burri *et al.*, 2017). In addition to the obtained results for DPPH are in agreement with the phenol contents determined for each sample. Plant polyphenolics used as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups (Aberoumand and Deokule, 2008). As the concentration of phenolic compounds or degree of hydroxylation of the phenolic increases, their DPPH radical scavenging activity also increased. (Mohdaly *et al*., 2011). Another study investigated that *S. oleraceus* and *S. arvensis* extract could be used as a potential source of natural antioxidant activities in scavenging of hydrogen peroxide and superoxide radicals as well as hydroxyl radicals, which may be due to the presence of polyphenolic constituent (Yin *et al*., 2007; Khan *et al*., 2012). The activities for these plants may be due to several compounds were identified such as Luteolin-O-glucuronide ,2-Pentadecanone, hydroxycoumarins and n-hexadecanoic acid in the aqueous extract of S. oleraceus (Krishnan *et al*.,2016; Banaras *et al*.,2020). While the extract of *C. pumilum* has sesquiterpene, caffeic acid, cichoriin, esculin, umbellic-ferone, scopoletin and 6,7-dihydroxy coumarin, flavone derivatives (cichoric acid, chlorogenic acid, apigenin, quercitin) (Mares *et al*.,2005; Rehman *et al*.,2014; Aisa *et al*.,2020). However, the antioxidant activity of *p. oleracea* extracts due to its content of phenolic compounds (El Kashef *et al*., 2018). In the same concern, Okafor and Ezejindu, (2014) found a high yield of saponin (32%) and alkaloid (26%) in *P. oleracea*. The medicinal values of these economically important plant species are due

to the presence of some chemical components which produce definite physiological action due to alkaloids, tannins, flavonoids and saponin etc. (Edeoga *et al*., 2005). In recent years, many alkaloids such as oleracone and oleracimine have anti-inflammatory bioactivities. Also, alkaloids are known for their pharmacological activities used for anticancer, antibacterial while Terpenoids have been implicated as antibacterial (Iyekowa *et al*.,2012). *P. oleracea* contains a different quantity of phenols, flavonoids, carbohydrates, alkaloids, tri terpenes, phenolic acids, ascorbic, malic, citric and oxalic acids (Liu *et al*.,2000). The most important compounds identified in the genus cichorium were polyphenolic acids, flavonoids, coumarins, lignans and terpenoids including (sescviterpene lactones, triterpenes). Other compounds present in chicorium were saponins, volatile compounds, amino acids, vitamins, fatty acids, carbohydrates, organic acids, phytosterols, small amounts of alkaloids and minerals (Das *et al.,*2016). Previously reported that the methanol extracts of some plants were more effective on most of the tested microorganisms than were the other extracts (Lin *et al*.,1999). Our results indicate that the antimicrobial component of the three plants may more soluble in ethanol and methanol as compared to water. The type of solvent also affects the physical properties of the extracts, especially the solubility of bioactive components. Different solvents soluble phytocomponents in different amounts, therefore, they have different antimicrobial activities. In the present investigation, all solvent extracts exhibited antimicrobial activity, but the ethanol and methanol extracts recorded much higher activity than both acetone and water extracts. other researchers have declared that aqueous extracts had the highest antimicrobial activity against a number of microorganisms (Faiku *et al*.,2016). There are many factors affecting antimicrobial activity. For example, the plant material, the mode of the extraction process and the extraction conditions (time, extraction temperature, pH, etc.) may significantly influence the solvent activity (Das *et al*.,2010 and Harbourne *et al*.,2013). The flavonoid apigenin was isolated from the aerial part of *P. oleracea L*. has antioxidant, anticarcinogenic, and spasmolytic activities and can reduce high blood pressure. That is why leaves of *P. oleracea L. are* a good source of apigenin, which can be added to food as a kind of functional ingredient or used as a vegetable, which has many beneficial effects on human health. It can also be used in medicine in standard forms of administration, such as capsules, tablets, and oral suspensions (Engelmann *et al.*,2002).

Conclusion

The plant extract used in this study is a good source of many effective compounds that are easy to use with many products especially these plants can be obtained in an easy, environmentally friendly, safe, and low-cost way had the greatest effect as an antifungal.

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