International Journal of Sciences

Research Article Volume 11 – October 2022 (10)

In-vitro Antifungal efficacy of Some Medicinal Plants

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Abstract: The aims of the present study are to examine *in-vitro* antifungal activity of crude extracts of Sudanese medicinal plants against two fungal species in order to verify their possible inhibitory activity and to identify the bioactive compounds responsible for the observed activity. Minimum inhibitory concentration (MIC) and phytochemical screening were investigated for the most active extracts to detect the active group of secondary metabolites. Agar diffusion method was used to test their sensitivity. Chloroform, methanol and aqueous extracts of a total number of 23 plants belonging to 19 genera and 17 families were investigated against *Saccharomyces cerevisiae* and *Candida albicans*. Among them, seven plant extracts showed efficacy against at least one of the two fungal cultures, and the methanol extracts of the different plants species exhibited a well marked antifungal activity. The bark methanolic extract of *Terminalia arjuna* (Combretaceae), gave the lowest minimum inhibitory concentration (MIC) value (4.25 µg/ml) against *C. albicans*, whereas the stem methanolic extracts of *Anogeissus schimperi* (Combretaceae), gave the lowest MIC value (0.18 µg/ml) against *S. cerevisiae*.

Keyword: Combretaceae, Inhibitory Effects, *Candida albicans*, *Saccharomyces cerevisiae*, MIC

Introduction:

Fungi cause a variety of infectious diseases which have profound impact on public health (Rodrigues & Nosanchuk 2020). Treatments of these fungal diseases are hindered by limited reliable diagnostic methods for a number of species (Wickes & Wiederhold 2018), restricted therapeutic options for few classes of drugs that are associated to both intrinsic and acquired resistance (Robbins *et al.* 2017), toxicity and unaffordable (Mourad & Perfect 2018). These factors justify the search for a novel bioactive natural products with different mode of actions (Di Santo 2010) and new control strategies (Vandeputte *et al*. 2012).

Natural products derived from animals (Gomes *et al*. 2021) or plants (Vila *et al*. 2013) continue to be important agents with therapeutic potentials. Antifungal properties have been investigated with increasing frequency in several families of the plant Kingdom (Cowan 1999), and various medicinal plants have been considered rich source of antifungal agents and can be used to treat fungal infections (Sepahvand *et al*. 2017).

Medicinal plants have been investigated in various regions worldwide to exhibit antifungal properties (e.g. Garcia *et al*. 2006, Nigussie *et al*. 2021) and a wide varieties of bioactive secondary metabolites were reported to possess in-vitro antifungal properties such as alkaloids, flavonoids, saponins, tannins and terpenoids (Arif *et al.* 2009) and phenolic compounds (Simonetti *et al.* 2020).

The aims of the present study are to evaluate *in-vitro* the potential antifungal activity of crude extracts of Sudanese medicinal plants against fungal cultures in order to verify their possible inhibitory activity and to recognize the phytochemical identity of the active groups responsible for the observed activity.

Materials and Methods:

1. Plant Materials

Various plant organs from 23 species belonging to 19 genera and 17 families, used in traditional medicine in Sudan were investigated. The Latin names of these plant species were updated according to POWO (2021). A voucher specimen for each sample of plant material was deposited at the Herbarium of Medicinal and Aromatic Plants Research Institute, National Centre for Research (Khartoum, Sudan). The plant names, families, and the parts used in the study are given in Table 1.

2. Extraction of plant materials for Antifungal Bioassay

The dried and ground parts of each plant material (200 g) were extracted sequentially with chloroform and methanol using a soxhlet apparatus. The extracts were concentrated under reduced pressure, using rotatory evaporator and the quantities thus obtained are shown in Table 1. Each dried extract was redissolved in either methanol: pet. Ether (2:1) or methanol and adjusted to give a final concentration of 50 mg /ml in preparation for the antifungal assay. Aqueous extract for each dried ground plant (15 g) was prepared from new batches of plant material by infusion method using boiled distilled water. The concentration of aqueous extracts was calculated in terms of crude powder before extraction as W/V.

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Published at: http://www.ijsciences.com/pub/issue/2022-10/
DOI: 10.18483/ijSci.2615; Online ISSN: 2305-3925; Print ISSN: 2410-4477

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3. Sensitivity tests:

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antifungal activity of the prepared extracts by means of the size of inhibition zones on the agar plates (Kavanagh, 1972).

Two ml of the standardized fungal stock suspension (108 –109) colony forming units: per ml) were thoroughly mixed with 250 ml of the sterile melted sabouraud dextrose agar which was maintained at 45° C for twenty ml aliquots of the inoculated sabouraud dextrose agar were distributed into sterile Petri dishes. The agar was left to set, and in each of these plates, four cups (10 mm in diameter) were cut using sterile cork borer (No. 4) and the agar disc were removed. Alternate cups were filled with 0.1ml each of the extracts, using standard Pasteur pipettes, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position, at 25° C for one day for the yeast. After incubation, diameters of resultant growth inhibition zones were measured averaged and the mean values were tabulated. Zone diameters were measured to the nearest millimetres. The interpretation of the inhibition zone diameters was based on the following data. The action of the tested organisms to each plant extracts were reported as "Sensitive" (Zone diameter >18 mm), "Intermediate" (Zone diameter =14-18 mm) or "Resistant" (Zone diameter <14 mm) (Brown and Blowers, 1978).

4. Minimum Inhibitory Concentration of the Crude Extracts

The minimum inhibitory concentration (MIC) of the most active crude extracts of the methanolic and aqueous extracts against fungal standard organisms were determined by the agar dilution method. A series of diluted plates were prepared. In these experiments, one loopful of culture was streaked on the plate and incubated at 25° C for seven days for the fungi (Kavanagh 1972). The results are shown in table 4. Nystatin and Clotrimazole were used as positive control for fungi cultures.

5. Fungi tested:

The plant extracts were tested against two fungal pathogens namely: *Saccharomyces cerevisiae* and *Candida albicans*. These fungi were supplied by the National Collection of type Cultures, London, U. K for (*S. cerevisiae* NCTC 10716) and American Type Culture Collection, Rockville, Maryland, USA for (*C. albicans* ATCC 7596).

6. Preliminary Phytochemical screening of the most active extracts:

A phytochemical screening of the most active and moderate samples were carried out to reveal to some extent the type of chemical constituents present. Investigation for the presence of secondary plant constituent was carried out using the standard methods (Farnsworth, 1983). The results were present in Table (3).

(50g) of the powdered plant material was refluxed with 250 ml of 80% ethanol for 4 hours. The cooled solution was filtered and more ethanol (80%) was passed through the mark to adjust the volume to 500 ml. This prepared extract was used for the unsaturated sterols, triterpenes, alkaloids, flavonoids and tannins tests. While for saponins and anthraquinones glycosides detection, fresh samples were used.

7. Percentage yield of the plant extracts:

The percentage yield of the different extracts examined was determined as percentages of the weight of the extracts to the original weight of the dried sample used.

Results

The preliminary investigation of 23 Sudanese medicinal plants belonging to 19 genera and 17 families for antifungal sensitivity against standard organisms namely; *C. albicans* and *S. cerevisiae* were depicted in Table (1).

On the basis of results performed in the present study using the Nystatin and Clotrimazole as reference antifungal agents (Table 2) against *C. albicans* and *S. cerevisiae*, the total number of extracts examined for sensitivity against the two standard organisms used was 75 extracts. Of these extracts 37 (49.33%) extracts were sensitive against one or both organisms with differences in potency, whereas 38 (50.66%) extracts were devoid of any activity against the two tested organisms.

The number of chloroformic extracts screened for sensitivity was 25, out of which 10 (40 %) extracts exhibited sensitivity against one or two of the fungi.

The total number of methanol extracts examined were 25, out of which 20 (80 %) showed sensitivity against one or two of the tested organisms.

Out of the 25 aqueous extracts, 7 (28 %) showed sensitivity against the *C. albicans* and *S. cerevisiae*.

It is evident that the methanol extracts of the different plants exhibited a well marked antifungal **activity** and Out of the most active 15 extracts examined, only 4 extracts were highly active against C. albicans while 11 extracts were active against S. cerevisiae. This indicated that S. cerevisiae is more susceptible than C. albicans to the action of the different extracts tested.

The most sensitive plants are *Anogeissus schimperi*, *Combretum pentagenum*, *Terminalia arjuna* (Combretaceae), *Bergia suffruticosa* (Elatinaceae), *E. scordiifolia* (Euphorbiaceae), *Hydnora abyssinica* (Hydnoraceae) and *Jussiaea erecta* L. (Onagraceae).

Phytochemical studies of the most active and moderate plant species revealed remarkable presence of flavonoids, tannins and saponins. Sterols and/or triterpens and alkaloids are present in low concentrations, Saponins and alkaloid were also present in some plants, whereas anthraquinones and cyanogenic glycosides were not detected.

The results obtained revealed that, most active extracts inhibited *C. albicans* growth with the minimum inhibitory concentrations in the range of 4.25 – 13.72 µg /ml. whereas most extracts active inhibited *S. cerevisiae* growth with the MIC in the range of 0.18 – 1.87 µg /ml (Table 4).

The results obtained revealed that, most extracts inhibited fungal growth (MICs) in the range of 0.25 - 4.0 µg/ml (Table 4).

Table (1) Preliminary screening for antifungal activity of some Sudanese plants

Family/ botanical name	**Part used**	**Solvent used**	**Yield%**	**Test organisms used*/M.D.I.Z.mm***	
				C. alb.	*S. cer.*
Aizoaceae *Aizoon canariense* L.	W	$CHCl_3$ MeOH H_2O	0.9 4.5 13.1	- 12 -	- 13 -
Molluginaceae *Glinus lotoides* L.	W	$CHCl_3$ MeOH H_2O	2.3 3.8 11.3	15 - -	17 - -
Asteraceae *Echinops longifolius*A. Rich.	W	$CHCl_3$ MeOH H_2O	2.5 3.8 5.8	11 13 -	14 16 -
Azollaceae *Azolla nilotica* Lam.	W	$CHCl_3$ MeOH H_2O	0.4 1.4 5.9	- 11 -	12 15 -
Chenopodiaceae *Salsola imbricate* Forssk.	W	$CHCl_3$ MeOH H_2O	2.1 7.8 14.5	- - -	11 12 -
Ceratophyllaceae *Ceratophyllum demersum* L.	W	$CHCl_3$ MeOH H_2O	1.1 1.5 4.1	- - -	- - -
Combretaceae *Anogeissus schimperi* Hochst. ex. Hutch. & Dalziel	F	$CHCl_3$ MeOH H_2O	1.5 6.7 7.1	15 20 13	17 21 14
Combretaceae *Combretum pentagenum* Vent.	L	$CHCl_3$ MeOH H_2O	4.1 14.7 9.1	- 19 14	- 25 15
	St	$CHCl_3$ MeOH H_2O	2.3 10.2 7.3	- 15 13	- 23 15
Combreataceae *Terminalia arjuna*(Roxb.) Wight. & Arn.	B	$CHCl_3$ MeOH H_2O	1.5 20.6 11.7	- 19 15	13 18 13
Elatinaceae *Bergia suffruticosa* (Del.) Fenzl	W	$CHCl_3$ MeOH H_2O	3.8 9.4 8.1	- 16 -	15 25 -
Euphorbiaceae *Euphorbia cuneata* Vahl	W	$CHCl_3$ MeOH H_2O	3.6 2.4 6.2	11 14 -	13 20 -
Euphorbiaceae *Euphorbia scordiifolia* Jacq.	L	$CHCl_3$ MeOH H_2O	10.4 35.5 8.7	15 17 14	18 26 19
	St	$CHCl_3$ MeOH H_2O	4.9 15.4 9.2	- 13 -	14 18 -
Euphorbiaceae *Euphorbia thi* Schweinf.	W	$CHCl_3$ MeOH H_2O	8.8 4.6 12.4	- 14 -	- 16 -
Euphorbiaceae ***Jatropha aceroides (Pax & Hoffm.) Hutch.***	St	$CHCl_3$ MeOH H_2O	1.4 5.2 14.2	- - -	- 15 -

Fabaceae *Tephrosia purpurea* (L.) Pers	W	$CHCl_3$ MeOH H_2O	1.3 4.0 6.5	- 13 -	11 18 -
Hydnoraceae *Hydnora abyssinica* A. Braun.	Rh	$CHCl_3$ MeOH H_2O	3.5 15.0 14.4	12 15 12	14 26 15
Lamiaceae *Leucas urticifolia* (Vahl) Benth.	W	$CHCl_3$ MeOH H_2O	o.5 o.3 8.8	- 11 -	14 15 -
Lamiaceae *Plectran barbatus* Andr.	W	$CHCl_3$ MeOH H_2O	2.6 5.5 8.2	- 13 -	- 19 -
Mimosaceae *Neptunia oleracea* Lour	W	$CHCl_3$ MeOH H_2O	2.0 2.5 3.1	- 11 -	12 15 -
Najadaceae *Najas pectinata* (Parl) Magnus	W	$CHCl_3$ MeOH H_2O	1.6 26.3 6.5	- 12 -	17 18 -
Onagraceae *Jussiaea erecta* L.	W	$CHCl_3$ MeOH H_2O	2.3 39.8 9.22	- 20 12	15 27 17
Onagraceae *Jussiaea repens* L.	W	$CHCl_3$ MeOH H_2O	1.2 6.6 4.9	- 15 -	- 20 -
Polygalaceae *Polygala irregularis* Boiss.	W	$CHCl_3$ MeOH H_2O	4.29 10.31 8.98	- - -	13 - -

C. alb.= Candida albicans; S. cer.= Saccharomyces cerevisiae

***** Mean Diameter of Growth Inhibition Zones, in mm. Values are the mean of 4 replicates.

- = no inhibition.

B= bark; F= fruit; L= leaves; R= roots; Rh= rhizome; S.= stem; W = whole plant.

Table (2) Antifungal activity of reference drugs against standard organisms

Drug	**Concentration**	**Test organism used* M. D. I. Z mm**	
		C. albicana	***S. cerevisiae***
Nystatin	500 µg/ml 50 µg/ml 25 µg/ml 12.5 µg/ml	32 28 26 23	31 26 24 21
Clotrimazole	20 µg/ml 10 µg/ml 5 µg/ml	43 33 30	21 19 15

Table (3) Preliminary phytochemical screening of most active tested plants

Botanical Name	Part used	**Sterols and/or** Triterpenes	**Alkaloids**	**Flavonoids**	**Tannins**	**Saponins**	**Anthraquinones**	**Cyanogenicglycosides**
A. schimperi	Fr	+	-	+	++	++	-	-
	ST	□	-	+++	++	□	-	-
C. pentagenum	L	++	-	++	+++	□	-	-
	ST	++	-	□	++	+	-	-
T. arjuna	B	□	-	-	++++	++	-	-
B. suffruticosa	W. P	±	-	++++	++++	+	-	-
E. scordifolia	L	++	+	±	+++	-	-	-
	St	-	±	±	+++	±	-	-
H. abyssinica	Rh	++	-	-	++++	+	-	-
J. erecta	W. P	+	-	++	++++	-	-	-

- = Not detectable; ± = traces; + = low concentration; ++ = medium concentration; +++ =high concentration; ++++ = v. high concentration.

L = Leave; B = Bark; Fr = Fruit; St = Stem; W. P = Whole Plant ; Rh = Rhizome.

Table(4) Minimum inhibition concentration (MIC) mg/ml of plant extracts against standard fungal organisms.

Botanical Name	Part used	Solvent	*C. albicans*	*S. cerevisiae*
A. schimperi	St	MeOH	7.93	0.18
		H_2O	8.81	0.55
C. pentagenum	L	MeOH	5.57	0.34
		H_2O	6.52	0.20
	St	MeOH	8.25	0.51
		H_2O	13.72	0.21
T. arjuna	B	MeOH	4.25	0.23
		H_2O	8.59	0.53
B. suffruticosa	W. P	MeOH	4.70	0.58
E. cuneata	W. P	MeOH	nd	0.60
	L	MeOH	8.87	0.47
H. abyssinica	Rh	MeOH	7.50	1.87
J. erecta	W. P	MeOH	9.46	0.59
		H_2O	23.07	1.08

L = Leave; B = Bark; Fr = Fruit; St = Stem; W. P = Whole Plant; Rh = Rhizome; nd = not detected

Discussion and Conclusions:

Seven species were recognized as the most active plants against one or both organisms (*C. albicans* and *S. cerevisiae*) using the agar diffusion method adopted by (Kavanagh 1972). This method although it was old, is still widely used as a tool to evaluate the sensitivity of micro-organisms to plant extracts (Barnard 2019). Three of the plants exhibiting activity, belong to the family Combretaceae (*Anogeissus schimperi*, *Combretum pentagenum*, *Terminalia arjuna*), and one each of the rest of the families: Elatinaceae, Euphorbiaceae, Hydnoraceae and Onagraceae. It is worth mentioning that all the species examined in the present study belonging to the family Combretaceae showed clear activity, against one or both organisms examined.

In addition, the bark extracts of *Terminalia arjuna* (Combretaceae) gave the lowest minimum inhibitory concentration for *C. albicans* (MIC value 4.25 µg /ml.) while the *Anogeissus schimperi* stem (Combretaceae) gave the most lowest MIC against *S. cerevisiae* (MIC value 0.18 µg /ml.).

This is in good agreement with previous studies (e.g. Baba-Moussa *et. al.* 1999, Batawila *et al.* 2005, Masoko *et al.* 2007, Fyhrquist *et al.* 2008), who investigated various species of Combretaceae for their antifungal activity.

Phytochemical screening of the most active antifungal plants revealed that they are particularly rich in tannins, flavonoides and saponins. These bioactive chemical compounds were previously correlated with this antifungal activity. Latte & Kolodziej 2000, Mickymaray (2019) and Savarirajan *et al.* 2021 showed that tannins, flanonoides and saponins respectively were responsible for this antifungal activity. Among these bioactive chemical compounds Fyhrquist *et al.* (2008) highlighted that tannins could be attractive due to their low toxicity to human beings.

These results provide a sound scientific basis for the use of this plant for the treatment of fungal infections.

Acknowledgement:

The authors are grateful to Mohammed Salih and Mahgoub Oal (MAPRI) for their technical assistance and NCR for financial support of study.

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