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Evaluation of Sorghum bicolor, Parsley and Azadirachta indica Extracts as Natural Cytoplasmic Stains for Buccal Cytology Smears in Central Sudan

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ABSTRACT:

Background: Sudan is mainly an agricultural country with vast fertile lands, we used wide variety of agricultural products to obtain stains from Sorghum bicolor, Parsley and Azadirachta indica (neem) , to be used in histopathology lab.

Objective: The present study had evaluated the effects of Sorghum bicolor, Parsley and Neem (Azadirachta indica) leaves extracts on their ability to stain cytoplasm in buccal smear.

Material and Method: The study was conducted at Wad Medani College of Medical Sciences and Technology, Medical Laboratory Science, Department of Histopathology and Cytology, Gazira state, Central Sudan. This study was carried out during December 2020 to December 2021; we used six stains with different solutions.

Result:

Sorghum bicolor extracted with absolute ethanol and mordanted with *Hibiscus sabdariffa* demonstrated the highest cytoplasmic staining score (mean score >80%), outperforming alcoholic extraction without mordant. Nuclear staining using Mayer's hematoxylin showed higher compatibility with the mordanted *Sorghum bicolor* extract compared to the alcoholic extract alone. Parsley extract prepared using 100% ethanol produced satisfactory cytoplasmic staining (mean score approximately 70%). Boiled *Azadirachta indica* extract yielded superior cytoplasmic staining (mean score >75%) compared with saline and ethanol extracts, although saline extraction adversely affected nuclear staining quality.

Conclusion: The extraction of Sorghum bicolor by absolute ethanol with mordant Hibiscus sabdariffa (karkadh) , extraction of Petroselinum crispum (Parsley) by absolute ethanol and extraction of boiled neem in tap water could be a useful substitute to eosin for the demonstration of cytoplasm in cytology specimen (buccal smear).

Key words: Sorghum bicolor, Parsley and Azadirachta indica (neem)

Introduction: Sorghum bicolor is a member of grass family (Poaceae) originated from North-Eastern tropical Africa, has a red dye present in the leaf sheath and sometimes in adjacent stem parts [(1)].It grows in clumps that may reach over 4 meters. Sorghum is an important staple food and also an important feed grain a crop. It is used in beer making; it is also distilled to make a popular spirit and vinegar [(2)]. In Africa, the dye is used for goat-skin leather (Nigeria) in local weaving design (Sudan). In some West African countries, the dye can be extracted from the plant to color cloths, calabashes and as body pigment[(3)]. Sorghum plant residues are used extensively as materials for roofing, fencing, weaving and as fuel. The use of Sorghum bicolor leaf sheath as a remedy for anemia by traditional medicine healers is common in Nigeria particularly within the local people of the Yoruba and Hausa tribes [(4)]. It is used in arrow poisons. The red pigment is said to have antimicrobial and antifungal properties in opaque or colored end products[(5)].Parsley (*Petroselinum crispum*) is derived from the Greek for 'rock celery'is an herb belonging to the Apiaceae. Parsley is a leafy vegetable, rich in many biologically active compounds, it can be distinguished from other leafy green herbs by its unique aroma[(6)]. Parsley is sensitive to water stress,especially if it is planted in the summer and at the end of spring, and to increase production and improve quality, a permanent source of water should be provided [(7,8)]. Parsley has anti-inflammatory, antimicrobial, diuretic and hypoglycemic properties due to its content ofessential oil and phenolic compounds [(9)].*Azadirachta indica* (neem), species of the (Meliaceae) family is native to the Indian subcontinent. Tree provides material that is widely used in several areas including agriculture, veterinary medicine, therapeutic medicine and wood harvesting [(10)]. It has been used in Ayurvedic medicine formore than 4000 years due to its medicinal properties. The earliest Sanskrit medical writings refer to the benefits of Neem‟s fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani medicine, and is now being used in pharmaceutical and cosmetics industries [(11)].

There is a lack of standardized experimental evidence assessing and comparing different extraction methods of these natural dyes using uniform staining and scoring criteria in cytology specimens.

This study aims to address this gap by systematically evaluating standardized extracts of *Sorghum bicolor*, *Petroselinum crispum*, and *Azadirachta indica* as cytoplasmic stains for buccal smear cytology, using a defined scoring system and direct comparison with routine hematoxylin–eosin staining. The findings may contribute to the development of safe, low-cost, and locally sourced alternatives to eosin in routine cytopathology practice.

Materials and Method:

Study population: The study was conducted at Wad Medani College of Medical Sciences and Technology, Medical Laboratory Science, Department of Histopathology and Cytology ,Gazira state, Central Sudan. This study was carried out during December 2019 to December 2020, 6 sample stains with 6 natural dyes extract and 1sample stain with routine stain(Hematoxlyin and Eosin) as a control. The six natural dyes were (germinating sorghum converted it into powder which is locally called (Zureea). Then we used from it two solutions put them in containers, in one container we dissolved S.bicolor in absolute ethanol alcohol and incubated for 3 days, in the second container we dissolved S.bicolor and Hibiscus sabdariffa in ethanol absolute alcohol and incubated it for 10 days, then filtered them by filter paper, Parsley we dried it up in the shadow for one day, then we changed it into powder by a grinder ,then we took 30g of this powder and added them to 100ml of alcohol and incubated for one day, to be used as cytological stain, the sample stained for 30 min by H&E staining method. We made an extract from Neem in two ways: firstly, we took some leaves from neem tree and added water to them, then we boiled them in a tightly closed pot for two hours, until the color of the neem leaves changed to pale yellow. Then we put the extract in brown bottle at room temperature for one day. Secondly, we took neem leaves and put them in the sunshine for three days, then we changed these leaves to powder by using a grinder, and took 30g of this powder and dissolved to absolute ethanol, and put it in a brown bottle for one day, and 30g dissolved in normal saline also put it in a brown bottle for one day, then we were used all of extracted solutions as cytoplasmic stain, the sample stained for 30 min by H&E staining method).The data was analyzed by blind person who does not have a background about the natural dyes which we used in the research and gave score from 0 to 10.

Data analyses: Scoring analysis by blinded person does not have a background about the natural dyes which we used in the research.

Ethical consideration: The study approval taken from on each of researchers in MST taken from all participants before collection.

Quality control: Samples representing the normal and pathological level of buccal smear was used for assessment of the quality control. Result of the target morphological of the control was accepted.

Results:

The results of Sorghum bicolor stains were represented in table (1).The result showed that extraction of Sorghum bicolor+ mordant (hibiscussabdariffa) for cytoplasm gave higher score than the Sorghum bicolor with ethanol. The score of Nucleus with Mayer's hematoxylins with extraction of Sorghum bicolor+ mordant (hibiscussabdariffa) gave higher score than the score of Mayer's Hematoxylin with alcoholic extraction. On the other hand the result of Parsley stain was represented in table (2) which was extracted by immersion Parsley in 100% ethanol and gave cytoplasm stain**.** Finally the results of *Azadirachta indica* (neem) were represented in table(3).The results showed extraction of neem by boiling gave higher score than the extracted with normal saline and absolute ethanol, but the extraction of neem with normal saline affected on the score of nucleus which was stained by Mayer's Hematoxylin.

Table(1): Sorghum bicolor stains

Stain	Cytoplasm score	Nucleus score
Sorghum bicolor with Ethanol%	6	7
Sorghum bicolor+ mordant (hibiscussabdariffa)	8	9

Description:

The addition of *Hibiscus sabdariffa* as a mordant significantly improved both cytoplasmic staining intensity and nuclear contrast compared to ethanolic extraction alone.

Table(2): Parsley stain

Stain	**Cytoplasm score**	**Nucleus score with Mayer's Hematoxylin**
Parsley	8	8

Description:

Parsley extract produced consistent green cytoplasmic staining with satisfactory nuclear clarity when combined with Mayer's hematoxylin.

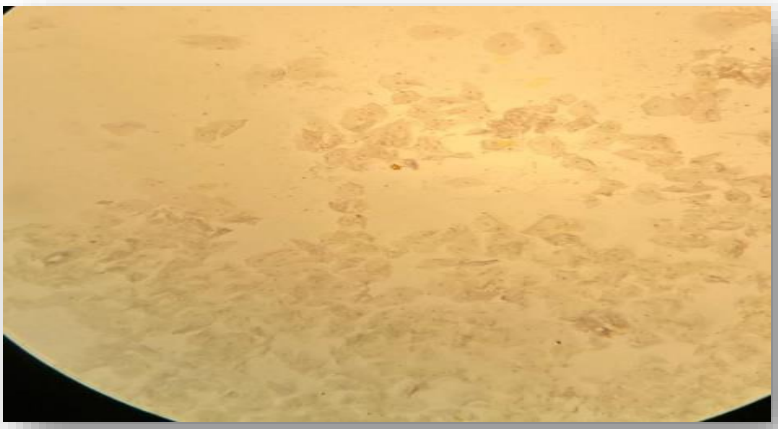
Table(3): Azadirachta indica (Neem):

Stain	Cytoplasm score	Nucleus score with Mayer's Hematoxylin
Neem with normal saline	6	7
Neem with ethanol	8	9
Neem boiling	9	9

Description:

The boiled aqueous neem extract demonstrated the highest overall staining performance, while saline extraction negatively affected nuclear contrast.

Figure (1): Sorghum bicolor with Ethanol



Figure(2): Sorghum bicolor+ mordant (hibiscussabdariffa)



Figure(3): Parsley in 100% ethanol

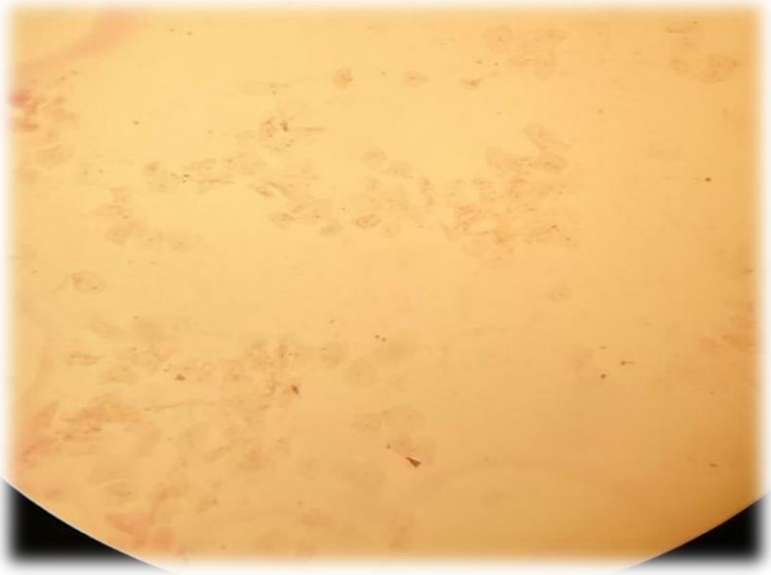


Figure (4): Azadirachta indica (neem) with normal saline



Figure (5): Neem with ethanol

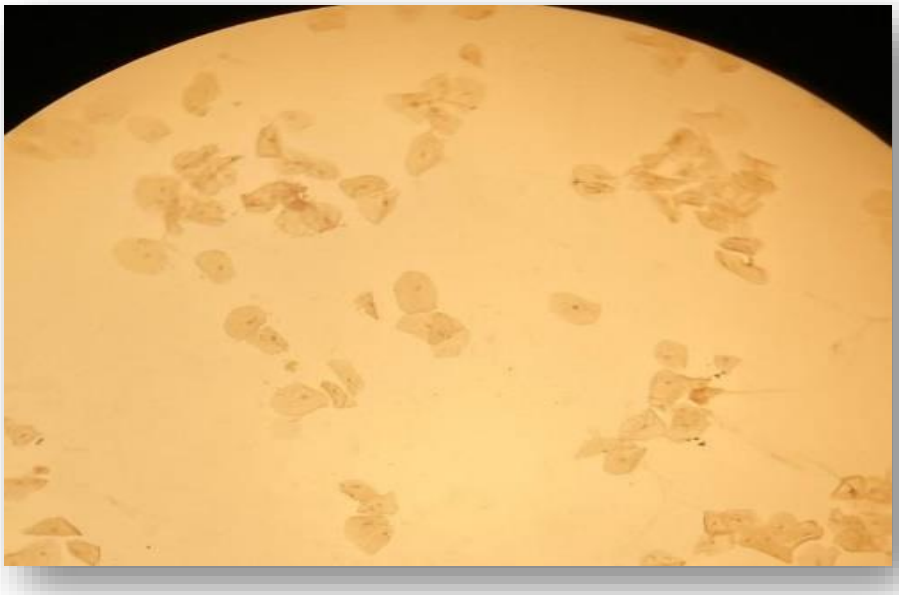
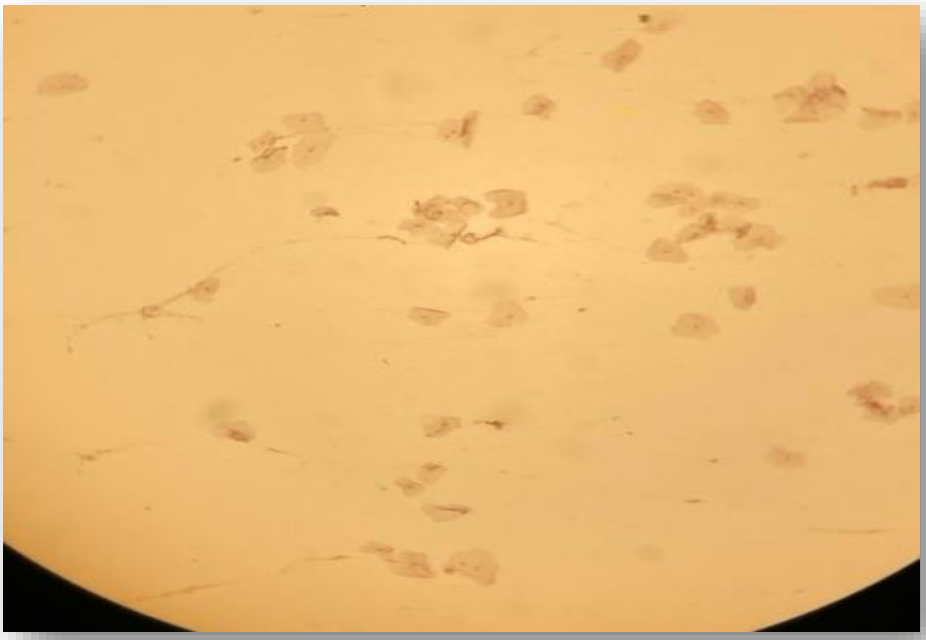


Figure (6) C:Neem boiling



Discussion:

In this study, we are sorted to using local natural alternatives dye that contribute to support the economy and reducing risks related to synthetic dye, and focus on the staining ability of Sorghum bicolor, Parsley and Azadirachta indica (Neem) that could substitute for eosin. Sorghum bicolor has been used extensively as food as a remedy for anemia, and medicines for several ailments, it has also been used as a dye for clothes, leather and calabashes. We work on Sorghum bicolor extract by absolute ethanolic alcohol, the solution gave us a dark red color, but when we filtered it we noticed it gave us a pale red color, and the color of cytoplasm gave a light pink color. Also we used ethanolic sorghum bicolor solution and added to it Hibiscus Sabdariffa (Karkadh) as one solution also gave light pink but best contrasted than ethanolic sorghum. Omoowo BT *et al*., 2014[1] work on the leaf extract to investigate the staining ability of the stalk of Sorghum bicolor as a counterstain on hematoxylin in tissue blocks of trachea, esophagus and ileum were retrieved from the autopsy block cytoplasm stain shades of pinkish brown. Bernard S A *et al*., 2015[12] to investigate the suitability of alcoholic extract of Sorghum bicolor stalk as a counter stain for Hibiscus extract stained nuclei to stain kidney and liver biopsies, and his result show nuclear component appear dark violet while cytoplasmic components appear light-brown. The point of disagreement between us, we used the solution on staining ability as cytopathological counter stain and did not add any material to the solution ,also we used ethanolic sorghum bicolor solution and added to it Hibiscus Sabdariffa (Karkadh) as one solution. Parsley has been used extensively as a table garnish worldwide, but its health-promoting uses are often ignored, and Hippocrates classified parsley as a diuretic. It has great demand in the food and cosmetic industries, We work on leaves of parsley of the plant and reported that ethanolic extract stained cytoplasm in shade of green. Navazio, J*et al.,*2012[6]. In Fatemeh Poureini*et al*., 2020 [13]study on Comparative the extraction of apigenin from parsley leaves. We work on leaves of Azadirachta indica (Neem) of the plant and reported that ethanolic, boiling, and normal saline extract stained cytoplasm in shade of green. Azadirachta indica (Neem) boiling extract has the good contrast color and gave higher score. These variations can be attributed to difference in the extraction solvents, concentration, temperature and PH. Azadirachta indica (Neem) has been used extensively as Larvicidal Activity, Antibacterial Activity, Antidiabetic evaluation, Antioxidant, Skin Disorders ,Anti-HIV/AIDS, Antiulcer, Antimalarial activity, Anti-tumor effect, Antifertility effect, Anti-dental caries, Antihypertensive and anti-hypercholesteremic effect. Imam Hashmat*et al.,* 2012[14].In PatelB. H, 2009[15] dyeing and antimicrobial finishing of polyurethane fiber with neem leaves extract. Extract was obtained by methanolic extraction method. The green color dye obtained from the

natural resource was used for dyeing of polyurethane fiber alone as well as in combination with various metallic salts. The point we disagreement between us, we used ethanolic, normal saline and boiling tab water extractions of Azadirachta indica (Neem)on staining ability as cytopathological counter stain.

Limitations

- Semi-quantitative scoring was based on a single evaluator.
- The study was limited to buccal smear cytology.
- No chemical characterization or pH standardization of extracts was performed.

Recommendations

- Future studies should include multiple blinded evaluators and inter-observer analysis.
- Chemical profiling of dye components is recommended.
- Evaluation of these stains on other cytology and histopathology specimens is warranted.

Conclusion:

The study concluded that, extraction of Sorghum bicolor with different additives and extractions gave good score for cytoplasmic stain, but the extraction by absolute ethanol with mordant Hibiscus sabdariffa (karkadh) which stained cytoplasm light pink gave best result, and nucleus stained with Mayer's hematoxylin. The extraction of Petroselinum crispum (Parsley) by absolute ethanol stained cytoplasm green, and nucleus stained with Mayer's hematoxylin. The extraction of Neem with different additives gave good score for cytoplasmic stain, but the extraction by boiled Neem in tap water which stained cytoplasm light green gave best resullt, and nucleus stained with Mayer's hematoxylin.

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