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FORMULATION AND EVALUATION OF ALTERNATIVE MICROBIAL CULTURE MEDIA TO AGAR-BASED MEDIA USING LOCAL PLANTS SEEDS

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ABSTRACT

Access to current microbiological culture media is characterized by some limitations including high cost, amongst others. This affects holistic learning, hence the need for formulation of alternative microbial culture media using local plants that are affordable, readily available, and accessible. This work sought to formulate and evaluate local plants seeds of *Brachystegia eurycoma* Harms, *Mucuna sloanei* Fawc and Rendle and *Detarium microcapum* Guill and Perr as alternative microbial culture media to agar-based microbial culture media. The seeds were processed and extracted using cold maceration method. *Staphylococcus aureus, Escherichia coli, Candida albicans and Aspergillus niger* were collected and sub-cultured to obtain pure isolates. Antimicrobial susceptibility test of the different pulverized plants seeds extracts was carried out using agar-well diffusion technique. Residual antimicrobial activities were inactivated using standard physicochemical and microbiological procedures. Microbial media were formulated from the antimicrobial-free pulverized seeds of methanol extract as gelling agents or agar replacement using pour plating method while microbial inoculation and colony count was carried using standard methods. Assessed textural and microbiological properties, gelling range (0.056 -0.07 g/mL) and time (3-40 min) of the inactivated pulverized seeds showed significant ($p<0.05$) varying properties compared to Nutrient agar (NA) and Sabouraud dextrose agar (SDA) as controls (0.028-0.056 g/mL and 18-30 minutes). The formulated culture media showed significant microbial growth in sabouraud dextrose broth than nutrient broth compared to control. Colony counts for *S. aureus* (1 ± 0.34) for *Mucuna. sloanei* and *Candida albicans* (5 ± 0.58) for *Brachystegia eurycoma* was less significant by a factor of 10 and 8 at $p<0.05$, within 24 and 72 hours compared to NA (15 ± 1.58) and SDA (43 ± 0.00). There was significant ($p<0.05$) colony count within 48 and 120 hours which was too numerous to count (TNTC) for bacteria and fungi compared to NA and SDA (TNTC). Therefore, the formulated media showed significant ($p<0.05$) comparative properties to agar-based microbial media with significant microbial growth and colony count. There is a feasibility of developing alternative media from either of the 3 plants to aid effective microbiological work in schools and laboratories.

Keywords*;* Formulation, Evaluation, Alternative Microbial Culture-media, Sabouraud dextrose agar.

1. INTRODUCTION

Microbiological culture media are special medium designed to support the growth of microorganisms. These media are prepared in solid, semi-solid or liquid form. The solid and semi-solid culture media contain a brown jelly-like substance known as agar while the liquid culture, called broth does not contain any agar. Microbial culture media is composed of nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals (for liquid media) and gelling agents (for solid media) essential for microbial growth (Uwa, *et al.*, 2018; Victor and Neftali 2018; Van der Horst *et al.*, 2007; Bridson and Brecker, 1970). Each component performs specific functions (Ackermann and Tardito, 2019). Their classifications also determine specific compositions, functions, type of cultures and examples (Saran *et.al.*, 2019; Sharma, 2017; (Rouf, *et al.*, 2017). Though numerous microbial growth media are in use (Daniel, 2013), they are characterized with limitations ranging from risk of contamination, reliability on well-designed primer set, no one definitive standard exist, no single-microbe-media- specificity, in fact many cannot grow in any known culture medium, time and resource intensiveness, unavailability and high cost. (Andrew, 2006; Adesemeye and Adedire, 2005). These limitations are serious problems for a developing country. Despite the fact that best practices for culturing new organisms have been developed, however, even with these best practices, the typical procedure for culturing new organism still requires a great deal of experience, and trial and error (Oberhardt, *et al.*, 2015).Culture media, as fundamentally important as it is in microbiological tests (Cundell, 2002) requires high quality specific to the growth and preservation of microbes, the possibility of achieving accurate, reproducible and repeatable microbiological test results and other usefulness may be reduced (Sandle, 2012). Unsuitable culture media can hinder the usefulness of micro-organisms in industry (Liu *et al.*, 2020; Mota-Gutierrez, *et al.*, 2019; Watzlawick and Altenbuchner, 2019; Heitmann, *et al.*, 2018), pharmaceutical (Cherukuri *et al.*, 2020; Fernandez-Cabezon, *et al.*, 2018; Rosenberg *et al.*, 2017; Liu *et al.*, 2016) and

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agriculture (Pham, *et al.,* 2019) which in turn affect research, learning and economic value. Agar (agarose gel) is used for the purpose of gelling (solidifying) the microbial culture medium. Agar is a gelatinous substance derived from sea weed. A cheap substitute for agar is guar gum, which can be used for its isolation and maintenance (Fattah, 2015). Very few studies have concentrated on replacing agar for solidification. In line with the other studies carried out in this area by a number of authors, some group of researchers (Ravanthies *et al*., 2012; Uthayasooriyan *et al*., 2016; Jannah *et al.,* 2021) have substances as alternative culture media. Even then, there is a necessity to formulate new media with appreciable gelling property and other components that is easily available using low-cost materials as substitutes for nutrient agar and sabouraud dextrose agar.

Brachystegia eurycoma (Harm) is a seasonal and well-known legume with its ethno-medicinal and nutritional values, popular among the people of the South eastern part of Nigeria (Okoli, *et al.,* 2015). It is an economic and woody plant belonging to the family Fabaceae and mostly found in the rain forest zone with attractive description (APG, 2016; Ndukwu, *et al*., 2017). In some states of Nigeria, *B. euyrocoma* is called achi in Igbo, 'akalado or eku' in Yoruba, 'akpakpa or taura' in Hausa, 'apauan' by the Ijaws, 'dewen' in Benin and 'odukpa' in Efik, Ibibio and Annang (Obochi, *et al.,* 2007). *B. eurycoma* is known to exhibit variety of activities (Ajuru and Okoli, 2013; Onyeso, *et al.,* 2016; Ndukwu, *et al*., 2017). It is used in the South eastern part of Nigeria as thickeners in local delicacies (Ajuru and Okoli, 2013). However, *B. eurycoma* has been grossly under-utilized despite the promise that it holds for food and drug development

Mucuna is a genus of around 150 accepted species of annual and perennial legumes of the family, Fabaceae, and of pan-tropical distribution with India as one of the natural centers in the world (Eilitta, *et al*., 2002; AGP, 2016). It is widespread in Africa from Sierra Leone east to DR Congo, and south to Angola, also in the Caribbean region, tropical America and islands of the Pacific Ocean (Jansen, 2005). In Nigeria, *Mucuna sloanei* are called different names at different places; 'ukpo' by the Igbos; 'karasuu' by the Hausas; 'yerepe' by the Yorubas and 'ibaba' by the Annang, Efik and Ibibio but horse eye bean in English (Obochi *et al*., 2007; Nwosu, 2012). The plants have been reported to possess useful constituents of high medicinal value of human and veterinary importance, and also constitute an important raw material in Ayurvedic and folk medicines (Ijeh *et al*., 2004; Sridhar and Rajeev, 2007; Nagatsua and Sawadab, 2009; Ojiako, *et al.,* 2012; Ejere *et al.,* 2015; Ekwe *et al.,* 2016). The seeds constitute a good source of several alkaloids, antioxidants, antitumor and antibacterial compounds (Adebowale and Lawal, 2003). In the South eastern part of Nigeria, *M. sloaneii* is used as thickeners in local delicacies (Ukachukwu *et al*., 2002). Regrettably, most of these plants are under-utilized, except as food gums, where the plant materials are used especially in Southeast Nigeria as food thickeners and stabilizers.

Detarium microcapum (Guil and Perr) is an African tree known as sweet detar, sweet dattock or tallow tree belonging to the family Fabaceae (AGP, 2016; Dayamba, *et al.,* 2016; Gaisberger *et al.,* 2017). It is an underutilized species of tree legume that grows naturally in the drier regions of West and Central African; from Senegal and Gambia east to Sudan and widely distributed in the semi-arid sub Saharan African which include Benin, Burkina Faso, Nigeria, etc, (Mariod *et al.,* 2009; Padonou *et al.,* 2015). *D. microcarpum* is one of the three species (*D. macrocarpum* and *D. senegalense)* of Detarium plant. *D. microcarpum* is called 'taura' in Hausa, 'kanuri' in Fulani, 'agashidam', 'etgsako' in Tiv, 'iyede' in Yoruba, 'ofor' in Igbo, Annang, Ibibio and Efik, (Iwu, 1993). *D. microcarpum* is known to contain bio-active and other substances for variety of uses by different parts; the fruits are edible and rich in vitamins C, the leaves and seeds are used for cooking, the root, stems bark are medicinal (Oibiokpa *et al.,* 2014). Despites its versatility (the numerous ethno-medical, industrial, pharmacological, etc uses), this species remains underutilized, especially its thickening potency has not been fully exploited. This begs for urgent need for its inclusion for further research work. Therefore, the objective of this work is to formulate and evaluate alternative microbial culture media to agar-based media using local plants-seeds of *B. eurycoma, M. sloanei, C. manni* and *D. microcapum*.

2. MATERIALS AND METHODS

2.1 Sample (Plants/Seeds) Collection and Identification

The seeds of *B. eurycoma, M. sloanei, C. manni* and *D. microcapum* were collected Nkwot Ikot Ebo in Etim Ekpo and Itam market in Itam, in Akwa Ibom State, Nigeria. The seeds were identified and authenticated by a taxonomist, Prof. (Mrs.) Margaret Bassey, in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Nigeria and with UUPH A32 (ZVii), A32 (ZVi) and A32 (ZVii) as leaf press made for *B. eurycome, M. sloanei* and *D. microcapum* as herbarium numbers in the Department of Pharmacognosy and Natural Product, Faculty of Pharmacy, University of Uyo. The shelves on the seeds were removed, air-dried for days and then milled with an electric milling machine before used for the analysis.

2.2. Seed Pulverization

2.3 The seeds of the different plants were pulverized into fine powder using hammer mill machine. The pulverized seeds were sieved to obtained particle sizes of 250-and-500 mm. The fine seed powders were separately stored in an air-tight container until needed for use for extraction and other activities. The fine seed powders were separately stored in an air-tight container until needed for use for extraction and other activities.

2.4 Preparation of Extract

Extracts of the seeds of *B. eurycome, M. sloanei* and *D. microcapum* were carried by cold maceration of the pulverized seeds using distilled water (aqueous), 70% ethanol, 90% methanol, and 99% dichloromethane for 24 and

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72 hours respectively with intermittent shaking. The liquid extracts were then filtered and concentrated at 40°C using rotary evaporator or water bath. The respective dried and crude extracts obtained were weighed differently and stored in a refrigerator for further studies (Sofoworo 1993; Trease and Evans, 2009).

2.4 Preparation and standardization of microbial culture

Microbial cultures such as *Staphylococcus aureus, Escherichia coli, Aspergillus niger* and *Candida albicans* were collected from the Departments of Microbiology and Pharmaceutical Microbiology and Biotechnology, Faculties of Sciences and Pharmacy, University of Uyo. The microorganisms were sub-cultured to obtain pure isolates by streaking on freshly prepared Nutrient agar (NA) and Sabouraud dextrose agar (SDA) media and incubated at 37°C for 24 hours (bacterial cultures) and at 28°C for 48 hours (fungal cultures) respectively (Collins and Lyne. 1979). Bacterial cultures were standardized to the turbidity of 0.5 Marfarland nephelometer standard corresponding to cells of approximate density ($1x10^8$ cfu/mL); while the fungal cultures were standardized using a haemocytometer to clear turbidity (Ekong *et al*., 2004).

2.5.1 Preparation of fresh Culture

2.5.1.1 Bacteria

The tested bacterial isolates namely; *Staphylococcus aureus* and *Escherichia.coli* were sub-cultured to obtain pure isolates by streaking on the freshly prepared NA medium from the original stock culture. The cultures were allowed to incubate at 37°C for 24 hours.

2.5.1.2 Fungi

In this study, *Aspergillus niger* and *Candida albicans* were also sub-cultured to obtain pure isolates by streaking on the freshly prepared SDA medium from the original stock culture. The cultures were allowed to incubate at (room temperature) 28°C for 48-72 hours (2-3 days).

2.6 Antimicrobial Activity of the Plants Seeds Extracts

The antimicrobial activity of the different pulverized plants seeds extracts was carried out using agar-well diffusion technique (AWD) (Magaldi *et al.*, 2004; Valgas *et al.*, 2007) on the standardized microbial inocula on NA and SDA plates, respectively. The residual antimicrobial (inhibitory) activity of the pulverized seeds was inactivated or neutralized following the standard physicochemical methods, with a bleaching agent, followed with continuous washing and standard dilution with water, prior to its usage as gelling agents and formulation of culture media (AOAC, 1990). After the physicochemical bleaching and washing, any residual antimicrobial activity assessment was again carried to ascertain and confirm total inactivation or nullification of antimicrobial activity in the pulverized plants seeds.

2.7 Media Formulation:

From the antimicrobial activity-inactivated or nullified pulverized plants seeds, sieving was carried out using 0.25 mm sieve size and kept in air-tight containers ready for use in solid and liquid media formulation.

2.7.1 Solid media; Two different solid media were prepared with specific amount (0.7-5.0 g) of each antimicrobial activity-inactivated or nullified pulverized plants seeds in a known quantity of distilled water, nutrient and Sabouraud dextrose broths, to check for its gelling time and other gelling parameters (consistency, transparency, pliability, smoothness, hardness and surface streaking ability). From the outcome of the result, the exact quantity of the pulverized plants seeds and the solvent systems (distilled water, nutrient and sabouraud dextrose broths) was ascertained. The exact excipients (organic and inorganic) as contained in the standard commercially formulated NA and SDA, were sourced for, alternatively the broths of those standard media was added in its right proportion to the exact quantity of the pulverized plants seeds showing significant gelling capacity to replace agar-agar while NA and SDA, served as control.

2.7.2 **Liquid Media:** The antimicrobial activity-inactivated or nullified pulverized plants seeds were continuously diluted and filtered using Whatman No-1 sieve apparatus or membrane filter apparatus. The filtrates obtained after autoclaving were used as the liquid media (broths) for the culture of the test organisms. Additionally, nutritional sources like those of NA and SDA, were added to the respective pulverized plants seeds filtrates as an alternative infusion media formulation.

These (solid and liquid media) were sterilized by autoclaving (Dixons, ST19T) at 121°C for 15 -20 minutes under the pressure of 15 Ibs/inch and were poured into sterile petri dishes and test tubes separately. The media were allowed to cool to a temperature of about 45°C and were dispensed into labelled plates. The gelling time was determined for different ratios of the formulated media. The properly gelled media was selected and used for culturing the microbial isolates. In all the experiments, the pH of the media was adjusted to 6.5 – 7$\pm$0.2 and 4.0$\pm$2.0 for bacteria and fungi respectively. Nutrient Agar and Sabouraud Dextrose Agar served as the positive control.

2.8 Microbial Inoculation into Formulated Media

2.8.1 Bacteria

Serial dilution was done according to the standard method to get a final bacterial inoculum concentration of 1.0 x 10^8 cell/mL. Bacterial suspension (0.1 mL) of the fresh, young and healthy standardized cultures of *Staphylococcus aureus* and *Escherichia coli* was taken using a sterile pipette inoculated onto the formulated pulverized plants seeds solid (alternative) media and NA (as control) by both pour and spread-plating techniques in triplicates (Collins and Lyne, 1979). Then all the plates were incubated at 37°C for 24-48 hours. After the incubation all the plates were observed for bacterial growth and the number of colonies was counted in the triplicate plates.

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2.8.2 Fungi.
Actively growing pure culture of *Aspergillus niger* and *Candida albicans* were inoculated onto the formulated plants-seeds solid (alternative) media and SDA (as control) by both pour and spread-plating techniques in triplicates (Collins and Lyne, 1979). All the inoculated plates were incubated at 28ºC for 72-120 hours. After, the inoculated plates were observed for fungal growth; and the number of colonies were counted and compared to those of the control to ascertain and compare the efficacy of the gelling capacity and nutritional properties of the pulverized plants seeds in formulation of culture media

2.9 Statistical Data Analysis
Data were reported as Mean ± SEM, of triplicate determinations. Statistical analysis was performed using GraphPad Prism version 5.0 for Windows. A two-way ANOVA was used to test for textural and microbiological properties among the formulated media and $p < 0.05$ was considered significant difference between test groups.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity and Inactivation
The pulverized plants seeds of the 3 plants showed variation in antimicrobial activity in the different extracts but after inactivation/bleaching with sodium hypochlorite and continuous washing with distilled water, filtering and drying for 24 h at room temperature, there was little or no antimicrobial activity especially with methanol extracts on *S. aureus, E. coli, A. niger* and *C.albicans* (Table 1) by *B. eurycoma, D. microcapum and M. sloanei* but the aqueous, ethanol and dichloromethane extracts showed slight activity. Therefore, for assessment of inactivation of antimicrobial activity, solvents extracts showed no antimicrobial activity in the sequence: methanol <aqueous <dichloromethane < ethanol. This became the basis why the research work was narrowed down to the usage of methanol extract only
.

Table 1: Gelling capacity of formulated culture media from inactivated antimicrobial activity of pulverized plants-seeds using nutrient broth as a based

Wt (g) in 30mL NB	*B. eurycoma* Gelling time	*M. sloanei* Gelling time	*D.microcapum* Gelling time	Control/ Gelling time NA	SDA
0.30	No gelling	No gelling	No gelling	Gels (30)	Gels (32)
0.45	No gelling	No gelling	No gelling	Gels (28)	Gels (25)
0.60	Partial gelling (>50)	No gelling	Partial gelling (>50)	Gels (25)	Gels (20)
2.10	Gels (30)	Gels (40)	Gels (35)	Gels (15)	Gels (12)

Key: Wt: weight, time in parenthesis () in minutes, NB (Nutrient broth).

Table 2: Gelling capacity of formulated culture media from inactivated antimicrobial activity of pulverized plants-seeds using sabouraud dextrose broth as a based

Wt (g) in 30 mL (SDB)	*B. eurycoma* Gelling time	*M. sloanei* Gellingtime	*D. microcapum* Gelling time	Control/ Gelling time NA	SDA
0.30	No gelling	No gelling	No gelling	Gel (28)	Gel (30)
0.45	No gelling	No gelling	No gelling	Gel (25)	Gel (27)
0.60	Partial gelling (>50)	No gelling	Partial gelling (>40)	Gel (22)	Gel (25)
2.10	Gel (35)	Gel (37)	Gel (32)	Gel (15)	Gel (18)

Key: Wt: weight, figures in parenthesis: (time), time (in minutes), SDB: Sabourad Dextrose Broth

3.2 Gelling Capacity and Formulation of Alternative Media
On formulation with varying concentrations, partial gelling was observed at concentrations 0.6 g/30 mL (0.02 g/mL) for *D. micrcoapum* and *B. eurycoma* only whereas all the 3 plants gelled at 2.1 g/30 mL (0.07 g/mL) using nutrient and sabouraud dextrose broths as their nutritive sources for both bacteria and fungi (Tables 1 and 2). But the gelling time was between 30 and 40 minutes as against 18 and 25 minutes for that of controls (NA and SDA). However similar gelling time for nutrient broth + agar was obtained when 1.5 g - 2 g of agar was added to different protein sources, which is consistent with the results obtained by Deivanayaki and Iruthayaraj (2012) for vegetable source and Annan-Prah *et al.,* (2010) for cowpea. About 3 g of sample was taken in the experiment which was carried out by Ravanthies *et al.,* (2012). *M. sloanei* had the longest gelling time of 40 minutes. Formulation with Nutrient broth (NB) showed significantly ($p<0.05$) less gelling time (30 minutes) compared to SDA (35 minutes) for *B. eurycoma.* Formulation with Sabouraud Dextrose broth (SDB) also showed significantly ($p<0.05$) less gelling time (37 mins) compared to NB (40 mins) for *M. sloanei* whereas SDB formulation base showed significantly ($p<0.05$) less gelling time (32 mins) compared to NB (35 mins) for *D. microcapum*, respectively. Therefore, the nutritive base or broth for this microbial culture media formulation follows the order; SDB > NB. Using nutrient and sabouraud dextrose broths was to determine or ascertain which broth will provide a better formulated microbial culture media with best antimicrobial activity. The pH was seen at the level of acidity but its adjustment may provide same or different results. The effect of particle size, complete dissolution of the pulverized plants seeds and homogenous mix before and after sterilization may have contributed to the

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delayed in gelling time and other differences observed in the morphological parameters. This is a challenge that needs to be solved as spotted in the course of this novel work.

Table 3: Microbial activity on formulated microbial culture and standard media

Incubation condition			**28ºC for 72 h**		**28ºC for 120 h**	
Plant	**Weight (g)**	**Based (60 mL)**	***A. niger***	***C. albicans***	***A. niger***	**C. *albicans***
B. eurycoma	5	NB	+	+	++	+
	6	SDB	+++	++	+++	+++
M. sloanei	5	NB	+	+	+++	+++
	6	SDB	++	++	+++	+++
D. microcapum	5	NB	+	++	++	++
	6	SDB	+	+	+++	++
Control	**(Sabouraud Dextrose Agar)**		+++	+++	+++	+++

Key: NB (Nutrient Broth), SDB (Sabouraud Dextrose Broth), (+) (growth), ++ (more growth), +++ (Too numerous to count).

Table 4: Microbial activity on formulated microbial culture and standard media

Incubation condition			**37ºC for 24 h**		**37 ºC for 48 h**	
Plant	**Weight (g)**	**Based (60 mL)**	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***
B. eurycoma	5	NB	+	+	+	+
	6	SDB	++	++	++	+++
M. sloanei	5	NB	+	+	+	+
	6	SDB	+	+	+++	+++
D. microcapum	5	NB	++	+	++	+++
	6	SDB	+	++	+++	+
Control	**(Nutrient Agar)**		++	++	+++	+++

Key: NB (Nutrient Broth), SDB (Sabourad Dextrose Broth), (+) (growth), ++ (more growth), +++ (Too numerous to count)
.

3.3. Microbial Inoculation into Formulated Media

Inoculating the bacterial isolates on the formulated microbial culture media, more microbial growth of *S. aureus* at 37ºC for 24 h and *E. coli* at 48 h of incubations was observed on *D. microcapum* formulated media and that of control at a concentration of 5 g/150 mL (0.03 g/mL) using nutrient broth based. But *B. eurycoma* and *M. sloanei* showed less growth (Table 3). Whereas in 6 g/150 mL (0.04 g/mL), more microbial growth of *S. aureus* and *E. coli* at 24 and 48 h was observed on *B. eurycoma* formulated media to that of control, but only that of *E. coli* on *D. microcapum* aligns with that of control on sabouraud dextrose broth. (Table 4).

From Table 3, SDB showed more growth (++) on *S. aureus* and *E. coli* for *B. eurycoma, E. coli* for *D. microcapum* which was same to that of control (Nutrient agar (NA), after 24 hours at 37ºC. NB favours more growth (++) of *S. aeurous* for *D. microcapum* same to that of control after 24 hours at 37ºC. Then SDB >NB and *D. microcapum* >*B. eurycoma* >*M. sloanei* compared to control (NA). In Table 5, SDB formulated antimicrobial culture media-base also showed same activity (growth too numerous to count +++) compared to control for *S. aeurous* and *E. coli* for *M. sloanei; S. aureus* for dichloromethane and *E. coli* for *B. eurycoma*. But only *E. coli* for *D. microcapum* after 48 hours at 37ºC showed same too numerous to count growth compared to control (NA). Hence, SDB >NB and *D.f microcapum* > *M. sloanei* > *B. eurycoma* compared to control (NA).

After 72 h at 28ºC incubation, SDB showed too numerous to count (TNTC) growth (+++) same as control on *A. niger* for *B. eurycoma* but less growth (++) on *A. niger; C. albicans* for *M. sloanei* (+) and *C. albican* for *B. eurycoma* (++) (Table 6). Also, SDB >NB and *B. eurycoma* > *M. sloanei* >*D. microcapum* compared to control (SDA). After 120 h SDB showed TNTC growth on *A. niger and C. albicans* (+++) for *B. eurycoma* and *M. sloanei* but only *A. niger* (+++) for *D. microcapum* significantly ($p<0.05$) compared to control (SDA) (+++). NB based formulation on the other end showed TNTC (+++) growth on *A. niger* and *C. albicans (+++)* for *M. sloanei*; and *A. niger* (+++) for *D. microcapum* respectively. Therefore, SDB >NB significantly ($p<0.05$) compared to control (SDA) and *M. sloanei* >*D.microcapum*>*B. eurycoma*. Also, at concentration of 5 g/150 mL (0.03 g/mL) incubated at 28ºC for 72 h, less fungal growth of *A. niger* and *C. albicans* were observed in *D. microcapum*, *B. eurycoma* and *M. sloanei* compared to that of control (SDA) with more growth. Whereas more growth of *C. albicans* was seen on *D. microcapum* formulated media but not significant ($p<0.05$) when compared to that of control (SDA). At 6 g/150 mL (0.04 g/mL), less microbial growth of *C. albicans* incubated at 28ºC for 72 h was observed in both *B. eurycoma* and *M. sloanei* formulated media but with more growth of *A. niger* on *B. eurycoma* formulated media only compared to that of control (SDA) (Table 5). At 28ºC for 120 h incubation, too numerous to count fungal growth of *A.*

niger and *C. albicans* were observed in both concentrations (5 g/150 mL (0.03 g/mL) and 6 g/150 mL (0.04 g/mL) on *M. sloanei* formulated media significantly ($p<0.05$) compared to control (SDA) (Table 6). Too numerous to count (TNTC) fungal growth was observed on *B. eurycoma* for both *A. niger* and *C. albicans* at 6 g/150 mL (0.041 g/mL).

Table 5: Colony counting for bacterial growth on formulated/standard media

Concentration	**5 g/150 mL**				**6 g/150 mL**			
Incubation condition	**37ºC for 24 h**		**37ºC for 48 h**		**37ºC for 24 h**		**37ºC for 48 h**	
Plants/Microbes	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***
B. eurycoma	8.00±0.58	18.00± 0.34	68.00±1.35	TNTC	36.00± 1.16	31.00± 0.88	68.00 ±1.35	TNTC
M. sloanei	1.00±0.34	3.00± 0.00	TNTC	TNTC	12.00± 0.58	21.00± 0.34	TNTC	TNTC
D. microcapum	17.00±0.88	5.00± 0.34	TNTC	TNTC	53.00± 1.10	42.0 ± 1.00	TNTC	TNTC
Control (NA)	15.00±1.58	19.00±0.48	TNTC	TNTC	54.00 ±1.00	41.00± 1.00	TNTC	TNTC

Key: TNTC (Too Numerous to Count): ($\geq$80), NA: Nutrient Agar, method of counting: (Physical counting)

Table 6: Colony Counting for Fungal Growth on Formulated/Standard Media

Concentration	**5 g/150 Ml**				**6 g/150 mL**			
Incubation condition	**28ºC for 72 h**		**28ºC for 120 h**		**28ºC for 72 h**		**28ºC for 120 h**	
Plants/Microbes	***A. niger***	***C. albicans***	***A. niger***	***C. albicans***	***A. niger***	***C. albicans***	***A. niger***	***C. albicans***
B. eurycoma	68.00±2.03	5.00± 0.58	TNTC	72.00±2.15	TNTC	32.00±0.88	TNTC	TNTC
M. sloanei	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
D. microcapum	19.00±0.91	21.00±0.58	TNTC	78.00±2.47	23.00±0.58	36.0±0.75	TNTC	TNTC
Control (SDA)	TNTC	43.00±0.50	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

Key: TNTC (Too Numerous to Count): ($\geq$80), SDA: Sabouraud Dextrose Agar, Method of counting: (Physical counting).

3.4. Microbial colony counts

Bacteria growth showed variable colony count (Table 5). At a concentration of 5 g/150 mL (0.04 g/mL), *D. microcapum* significantly ($p<0.05$) showed the highest colony count (17.00±0.88) even more than the control (15.00±1.58) after 37ºC for 24 h, followed by *B. eurycoma (*8.00±0.58) and with *M. sloanei* (1.00±0.34) as the least for *S. aureus.* Whereas, for *E. coli, B. eurycoma* (18.00±0.34) was more significantly ($p<0.05$) colony forming compared to control (19.00±0.48). *M. sloanei* and *D. microcapum* showed less significant ($p<0.05$) in colony forming compared to control at 37ºC for 24 h. At 37ºC for 48 h incubation, the bacterial growth was significantly ($p<0.05$) TNTC for *E. coli* and *S. aureus* except for *B. eurycoma* (68.00±1.35) (Table 5). Therefore, sequential order with highest colony forming inactivated pulverized plants-seeds formulated culture media follows: *D. microcapum* (17.00±0.88) > *B. eurycoma (*8.00±0.58) >*M. sloanei* (1.00±0.34) compared to control (NA) (15.00±1.58). Also at a concentration of 6 g/150 mL (0.04 g/mL) (Table 5), *D. microcapum* significantly ($p<0.05$) showed the highest colony count (53.00±1.10) almost the same as control (54.00±1.00) after 37ºC for 24 h, followed by *B. eurycoma* (36.00±1.16) and with *M. sloanei* (12.00±0.58) as the least for *S. aureus.* Whereas for *E. coli, D. microcapum (*42.00±1.00) was more of significant ($p<0.05$) colony count compared to control (41.00±1.00) followed by *B. eurycoma (31*.00±0.88) and with *M. sloanei* (21.00±0.34) as the least colony count. At 37ºC for 48 h incubation, the bacterial growth was significant ($p<0.05$) TNTC for *E. coli* and *S. aureus* except for *B. eurycoma* (68.00±1.35) (Table 5). Therefore, sequential order with highest colony forming inactivated antimicrobial pulverized plants-seeds formulated culture media follows: *D. microcapum* (53.00±1.10 and 42.00±1.00) > *B. eurycoma* (36.00±1.16) and (31.00±0.88) >*M. sloanei* (12.00±0.58 and (21.00±0.34) for both *E. coli* and *S. aureus* compared to control (NA) (54.00±1.00 and (41.00±1.00) at 6 g/150mL (0.04 g/mL) for 24 h. After 48 h, the sequences became; *D. microcapum* (TNTC and TNTC) >*M. sloanei* (TNTC and TNTC) >*B. eurycoma* (68.00±1.35) and TNTC) for both *E. coli* and *S. aureus* compared to control (TNTC and TNTC) at 6 g/150mL (0.04 g/mL). Fungal growth also showed variable colony count (Table 6). At a concentration of 5 g/150 mL (0.04 g/mL), *M. sloanei* significantly ($p<0.05$) showed same colony count (TNTC) with that of control (SDA) after 28ºC for 72 and 120 h followed by *B. eurycoma (6*8.00±2.03) and *D. microcapum (*19.00±0.91) for *A. niger*. Whereas for *C. albicans, M. sloanei* was significantly ($p<0.05$) TNTC compared to control (43.00±0.50). *D. microcapum* (21.00±0.58) showed significant ($p<0.05$) colony count compared to *B. eurycoma* (5.00±0.58) and that of control (43.00±0.50). At 37 ºC for 120 h incubation, the fungal growth was significantly ($p<0.05$) TNTC for both *A. niger* and *C. albicans* respectively. But *D. microcapum* (78.00±2.47) and *B. eurycoma* (72.00±2.15) also showed significant ($p<0.05$) colony count compared to that of control (TNTC). Therefore, sequential order with highest colony forming inactivated antimicrobial pulverized plants-seeds formulated culture media follows: *M. sloanei* (TNTC and TNTC) >*D. microcapum* (TNTC and 78.00±2.47) > *B. eurycoma* (TNTC

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and 72.00±2.15) compared to control (SDA) (TNTC and TNTC) for 120 h at 5 g/150 mL (0.033 g/mL). At a concentration of 6 g/150 mL (0.04 g/mL), *B. eurycoma M. sloanei* and *D. microcapum* significantly ($p<0.05$) showed same colony count (TNTC) with that of control after 28ºC for 72 and 120 h for both *A. niger* and *C. albicans* except for *D. microcapum* with colony count *(*23.00±0.58) for *A. niger* and (36.00±0.75) for *C. albicans* at 28ºC for 72 h (Tables 10).Therefore, sequential order with highest colony forming inactivated antimicrobial pulverized plants-seeds formulated culture media follows: *M. sloanei* (TNTC and TNTC) >*B. eurycoma* > (TNTC and (32.00±0.88) > *D. microcapum* (23.00±0.58 and *(*36.00±0.75) compared to control (SDA) (TNTC and TNTC) at 6 g/150 mL (0.04 g/mL).

Conversely, the formulated alternative liquid media (broths) did not give statistically significant ($p>0$) results as the filtrates were turbid and could not allowed for proper and clear result observation and differentiation of cultures after inoculation. The turbidity and disparity in results observed could be traced to some limitations such as presence and flocculation of tiny particles from the pulverized plants seeds due to incomplete polishing and dissolution in the solvent, usage of different batches of products, fluctuations in power supply during sterilization, as well as delayed take-off and enhancement of microbial activity due to malfunctioning and unavailability of certain apparatus and equipment. The findings from this work has directed attention from the usual usage of these plants for their nutritive, medicinal and other usages, to their plausible usages as alternative microbial culture media, owing to their relatively significant gelling property, nutritive value and microbial growth support. Hence, the comparative growth of microbes to NA and SDA observed from the experiment. Also, as already known, agar is devoid of nutritive value; hence an advantage of these plants over agar in culture media formulation. Further research is therefore recommended on seed pulverization/particle size reduction, polishing, use of different solvents and solubility optimization profiles of the test local plants pulverized seeds as well as other antimicrobial activity inactivation processes for an enhanced optimal alternative microbial culture media formulation and economy.

4. CONCLUSION

From the result of this research work, pulverized seeds of *D. microcapum*, *B. eurycoma* and *M. sloanei* proved to be utilizable as gelling agents in the formulation of microbial culture media, although its gelling potential is significantly ($p<0.05$) low comparative to that of agar agar. Though the initial incubation time or lag phase of the microbial growth and its colony count showed less activity on the formulated media compared the controls (NA and SDA), the performance in other phase showed better or more significance ($p<0.05$). Maximal activity was seen in *E. coli* and *S. aureus* in *B. eurycoma* and *D. microcapum* formulated culture media with significantly ($p<0.05$) higher growth rate compared to NA at 0.03 g/mL for 24-48 h at 37ºC incubation. At 0.04 g/mL concentration however, there was significant ($p<0.05$) maximal colony count as in that of NA. *A. niger* and *C. albicans* (72.00±2.15 and TNTC) grew well in the formulated culture media significantly ($p<0.05$) compared to SDA (TNTC). The present study clearly showed in comparison with NA and SDA the possibility of using *D. microcapum*, *B. eurycoma* and *M. sloanei* as alternative gelling agent or enrichment media for bacteriological and mycological studies. Hence, this serves as a direction for further research work in order to curb the problems of microbial culture specificity, consistency, unavailability, accessibility and affordability of microbial culture media for holistic learning, research and laboratory work. It is recommended for further research in the direction as listed in the discussion, to ascertain the suitability of using these plants seeds as alternative formulated culture media.

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