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Full Length Research Paper

Performance of the Panbio™ COVID-19 Ag Rapid Test in a health care setting in Ouagadougou, Burkina Faso

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This study aimed to evaluate the performance of the Panbio™ Covid-19 Ag Rapid Test (Abbott) in a medical center in Ouagadougou. The Panbio™ COVID-19 Ag test was evaluated from January 26 to March 31, 2021 in symptomatic and asymptomatic patients in the medical Centre of Kossodo. A total of 268 individuals were tested by both SARS-CoV-2 RT-PCR, and antigen RDT. Of these 268 individuals, 52 were positive and 216 were negative for COVID-19 RT-PCR. The performance parameters of the test and its Kappa agreement with the RT-PCR were calculated according to the presence or absence of symptoms in the patients on one hand, and according to the time onset of symptoms on the other hand. The sensitivity of the Panbio™ COVID-19 Ag Rapid Test ranged from 29.63% (95% CI: 13.75 to 50.18) among COVID-19 asymptomatic patients, to 87.5% (95% CI: 52.91 to97.76) among symptomatic patients with symptom onset time of 1-5 days. Similarly, the Panbio™ COVID-19 Ag Rapid Test specificity was 97.3% (95% CI: 90.58 to 99.67) and 96.4% (95% CI: 91.81 to 98.82) in symptomatic and asymptomatic RT PCR negative patients. The Panbio™ COVID-19 Ag Rapid Test shows good performance in detecting COVID-19 cases in patients with a symptom onset time of less than seven (7) days. This performance is even better when the symptom onset is reduced to five (5) days. The results show that the antigen RDT is not suitable for COVID-19 detection among asymptomatic patients.

Key words: COVID-19, SARS-CoV-2, diagnosis, antigen test, rapid test, point-of-care.

INTRODUCTION

COVID-19 has been a major public health problem for countries around the world since it emerged in December

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2019 in Wuhan, People's Republic of China (Wu et al., 2020). The SARS-COV-2 infection is undoubtedly one of the greatest pandemics that humanity has ever experienced. According to new estimates by the World Health Organization (WHO), as of 30 August 2022, the global epidemiology estimates 599,071,265 confirmed cases of COVID-19 and 6,467,023 deaths (WHO, 2021b). The African region, particularly Burkina Faso, seems to be relatively spared by the pandemic compared to the rest of the world (Wamai et al., 2021). In Burkina Faso, as of 31 July 2022, the number of COVID-19 officially reported cases was 21,204, including 387 deaths (https://covid19.who.int/table).

The current reference method for COVID-19 diagnosis is Real-Time Polymerase Chain Reaction (RT-PCR), which is specific for the detection parts of the SARS-CoV-2 genome and the virus responsible for COVID-19 (Carter et al., 2020; La Marca et al., 2020; Zhai et al., 2020). This diagnostic method is only available in laboratories equipped with a molecular biology technical platform such as RT-PCR thermocyclers. It is performed on nasopharyngeal or oropharyngeal swabs, sputum or bronchoalveolar lavage samples (Zhai et al., 2020). According to standard protocols, RNA (ribonucleic acid) must be extracted and its presence confirmed by RT-PCR (Carter et al., 2020; Zhai et al., 2020).

This requires several steps and sometimes about 48 to 72 h for the return of the results to the care staff, with the potential risk for further spread of the virus meanwhile. RT-PCR is the gold standard for detection of SARS-CoV-2 virus. The application of a rapid antigen detection kit is limited by its sensitivity (Mak et al., 2021)

Rapid tests for the antigen diagnosis of SARS-CoV-2 have been developed (Carter et al., 2020; Deeks et al., 2020; Dinnes et al., 2020; La Marca et al., 2020). They are easy to use outside the laboratory and provide results in less than 30 min. Rapid tests for the detection of SARS-CoV-2 antigens are recommended for use particularly in the diagnosis of COVID-19 in symptomatic cases, contacts of confirmed cases, outbreaks, and screening of high-risk workers such as health care workers. (Loho and Widodo, 2021; Sumita et al., 2018; Thakur et al., 2021; WHO, 2021a, 2021c; Yamamoto et al., 2021). It is expected that antigen tests with good clinical performance could be an alternative in the triage of symptomatic patients in health care settings, especially when access to RT-PCR is limited (WHO, 2021a). With the decentralization of SARS-CoV-2 diagnosis and infection control at land border crossings, West African countries, including Burkina Faso, are using RT-PCR (Sagna et al., 2021; Zoure et al., 2022) and increasingly the antigen testing in health centers and the surveillance of SARS-CoV-2 among travelers at land entry points. However, the performance of these tests evaluation has not been conducted in our context. This study proposed to evaluate the performance of the Panbio™ Covid-19 Ag Rapid Test (Abbott) to contribute to the strengthening of access to biological diagnosis of COVID-19.

MATERIALS AND METHODS

Study site

The study was conducted in Ouagadougou, prior to the introduction of the vaccine against SARS-CoV-2 infection in Burkina Faso. Participants were recruited at the “Centre Médical avec Antenne Chirurgicale” (CMA) in Kossodo, Ouagadougou.

Study type, period, and population

A cross-sectional evaluation of the Panbio™ COVID-19 Ag test was conducted between January 26 and March 31, 2021.

The study population consisted of males and females of all ages who were seen at the COVID-19 screening center of the Kossodo Medical Center with Surgical Branch (CMA) with or without symptoms who consented to participate in the study. Participants were selected according to the following criteria: (i) male or female of any age, (ii) voluntary and willing to be diagnosed with COVID-19, (iii) clinically suspected (symptomatic) or not of COVID-19. A subject suspected of having COVID-19 in an epidemic setting is defined as one with an acute onset of fever, cough or an acute onset of three or more of the following signs or symptoms: fever, cough, general weakness, fatigue, headache, muscle pain, sore throat, runny nose, difficulty breathing, lack of appetite, nausea, vomiting, loss of smell, diarrhea, mental disturbance and severe acute respiratory infection (SARI): with a history of fever (T°≥ 38°C) and cough; occurring within the last 7 days requiring hospitalization.

Not included in the study were (i) patients with active nose bleeds, or with facial injuries and trauma or a condition that creates a mechanical barrier to safely obtaining samples; (ii) patients enrolled in a study to evaluate an investigational drug or vaccine; (iii) patients with nasopharyngeal specimens collected within the last 24 h of enrollment and (iv) nasopharyngeal specimens collected more than 2 hours after patient enrollment.

Sampling and sample size

The authors enumerated patients meeting the above criteria (suspected COVID-19 disease cases) during the study period until the desired numbers of positive and negative tests were reached. A total of 268 individuals (symptomatic or not) were tested by both RT-PCR and antigen RDT. Of these 268 individuals, 52 were positive and 216 were negative for COVID-19 RT-PCR.

Recruitment of participants and on-site testing procedure

Recruiting

Patients’ recruitment was carried out by the providers (an investigator, a sampling agent, and a laboratory technician) of the COVID-19 disease screening site at the Kossodo medical center. At the site, participants were examined for COVID-19 disease symptoms using the national checklist for COVID-19 disease screening. For individuals who consented to participate to the study, two nasopharyngeal swab samples were simultaneously collected, (i) for the on-site Panbio COVID-19 Antigen RDT, (ii) in Viral Transport Medium (VTM) for the SARS-COV2 RT-PCR reference testing in the laboratory.

Nasopharyngeal swabs

Two nasopharyngeal swabs were taken from each patient at

inclusion. One of the swabs collection material was provided in the Panbio COVID-19 Antigen kit (for on-site antigen testing) and the other using the regular swab and viral transport medium (VTM) for routine RT-PCR reference testing in the laboratory.

Samples intended for RT-PCR were transported to the laboratory at the end of the day by the specialized service of the post office and stored at +4°C before being analyzed the same day following collection.

Panbio™ COVID-19 Ag

The Panbio™ COVID-19 Ag rapid test device is a lateral flow immunochromatographic test. It is a rapid in-vitro diagnostic test for the qualitative detection of SARS-CoV-2 antigen (Ag) in human nasopharyngeal swab specimens from individuals meeting the clinical or epidemiological criteria for COVID-19. The Panbio™ COVID-19 Ag Rapid Test Device is intended for professional use only and is intended to be used as an aid in the diagnosis of SARS-CoV-2 infection. The product may be used in any laboratory and non-laboratory environment that meets the requirements specified in the instructions for use and local regulations. The Panbio™ COVID-19 Ag Rapid Test is supplied as a cassette containing a lateral flow test strip and can be stored at 2°C to 30°C.

The Panbio™ COVID-19 Ag rapid test was used according to the manufacturer's instructions. Direct swab specimens were tested immediately at the health facility after collection. Panbio™ COVID-19 Ag external control swabs (positive and negative) were tested with a Panbio™ COVID-19 Antigen test each time a new kit was opened, for use.

RT-PCR of SARS-COV-2 in the laboratory

Nasopharyngeal samples taken in VTM tubes were used for routine RT-PCR of SARS-CoV-2, the reference method for confirmation of COVID-19 cases. RNA extraction with QIAamp Viral RNA Mini Kit (QIAGEN®) and amplification performed with kits made available to the laboratory by the Ministry of Health for the diagnosis of COVID-19 in Burkina Faso. The amplification and testing interpretation of SARS-CoV2 results were done using the STANDARD nCoV Real-Time Detection kit (SD BIOSENSOR, Inc. following the manufacturer's instructions, blinded to the RDT results. The presence of SARS-CoV-2 RNA indicates an ongoing COVID-19 infection. The Cycle Threshold for each sample was also collected to establish the viral load.

Origin of the tests

The Panbio™ COVID-19 Ag Rapid Test was provided by Abbott Diagnostics for evaluation and the RT-PCR test was provided by the Ministry of Health of Burkina Faso as part for the routine diagnosis of COVID-19 disease in Burkina Faso.

Data processing and analysis

Data were entered into Excel and analyzed using Open-Epi software ((http://www.openepi.com). For the Panbio™ COVID-19 Ag rapid test, the results obtained were compared with those of the RT-PCR, and its main performance characteristics were determined. For this purpose, the results of the Panbio™ COVID-19 Ag rapid test were classified into 2 categories (positive or negative results). Regarding, the known results of the RT-PCR method (a reference to the antigen RDT), the Ag-RDT results were classified into true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN) on a double-entry contingency table (Table 2). Test sensitivity was calculated according to the formula (TP)/(TP+FN) and diagnostic specificity according to the formula (TN)/(TN+FP). In addition to the two main characteristics (Sensitivity and Specificity) of the diagnostic performance of the test, other test-specific parameters such as positive predictive value (PPV) and negative predictive value (NPV): PPV = TP/TP+FP and NPV=TN/TN+FN); the positive and negative likelihood ratios (LR+ and LR-); and the Kappa Coefficient of agreement between the antigen RDT and the RT-PCR tests. These characteristics were calculated with their 95% confidence intervals. The results of these calculations were expressed as a percentage. The Kappa coefficient of agreement was interpreted according to the criteria of Landis and Koch (1977) (Landis & Koch, 1977) as follows: Kappa <0, no agreement; 0 < kappa≤ 0.2 = slight agreement; 0.2< kappa < 0.4 = moderate agreement; 0.4 <kappa≤ 0.6; moderate agreement; 0.6<kappa≤0.8 = substantial agreement; 0.8<kappa≤1, near-perfect agreement.

The results of antigen RDT evaluation are presented according to several situations: (i) The first according to the use of the Panbio™ COVID-19 Ag Rapid Test at any time regardless of symptoms, (ii) the second taking into account symptoms and time of onset in the diagnosis of suspected COVID-19 disease cases, and (iii) the third in COVID-19 disease asymptomatic patients.

RESULTS

A total of 268 participants were tested with both the RT-PCR and the Panbio COVID-19 Rapid Antigen Test. The mean age was 39.7 years with ranges from 9 to 86 years. Males represented 62.3% (167/268) of the participants. Among the participants, 61.9% were asymptomatic (166/268), while 36.9% (99/268) had symptoms. The presence or absence of symptoms was not reported in 1.2% (3/268) of the participants. For 29 of the 99 participants with symptoms, the reported symptom onset periods were 1 to 5 days, for 47 participants the period was 1 to 7 days. Finally, 9 symptomatic participants had a symptom onset time greater than 7 days, while for 14 symptomatic participants, the symptom onset time was unknown (Table 1).

Performance of Panbio™ COVID-19 Ag Rapid Tests

Tables 2, 3, 4, and 5 show the raw results and performance of the antigen tests in several situations compared with RT-PCR. It is shown that the sensitivity of the Panbio™ COVID-19 Ag Rapid Test ranges from 29.63% (95% CI: 13.75 to 50.18) among patients asymptomatic for COVID-19 disease, to 81.82% (95% CI: 52.3 to 94.86) in symptomatic patients with a symptom onset time of 1 to 7 days. Among patients with a symptom onset time of 1 to 5 days, and detected positive for SARS-CoV-2 by RT-PCR with a Ct value ≤ 33, the sensitivity of the Panbio™ COVID-19 Ag Rapid Test is estimated to be 87.5% (95% CI:52.91-97.76), compared to 80.0% in those with a symptom onset time of 1 to 7 days with a Ct ≤ 33. Overall, regardless of symptoms, the sensitivity is 50.0% (95% CI: 29.45 to 67.47) for Ct values ≤ 33, compared with 29.2% (95% CI: 10.69 to 44.87) when the Ct value was greater than 33 (Table 5).

Table 1. Socio-demographic characteristics of participants.

Characteristics	Number	%
Age (n=268)		
≤ 25 years	18	6.72
>25 years	130	48.51
Missing	120	44.78
Sex (n=268)		
Male	167	62.31
Female	101	37.69
SARS-CoV-2 RT-PCR results (n=268)		
Negative	216	80.60
Positive	52	19.40
Clinical status (n=268)		
Asymptomatic	166	61.94
Symptomatic	99	36.94
Missing	3	1.12
Symptoms onset (n=99)		
1-5 days	29	29.29
1-7 days	47	47.47
> 7 days	9	9.09
Missing	14	14.14
Total	268	100

Source: Authors

Of 27 asymptomatic patients who tested positive by RT-PCR, eight returned positive by Panbio™ COVID-19 Ag Rapid Test (29.6%). Among the 19 symptomatic patients who tested negative for Panbio™ COVID-19 Ag, 12 had a Ct value greater than 33, which is considered low contagious according to the literature (Al Bayat et al., 2021; Platten et al., 2021).

As for the specificity of the Panbio™ COVID-19 Ag Rapid Test, it was 97.3% (CI95%: 90.58 to 99.67) in symptomatic patients and 96.4% (95% CI: 91.81- 98.82) in RT-PCR negative asymptomatic patients.

DISCUSSION

Several rapid antigenic diagnostic tests for COVID-19 disease have been developed since the appearance of SARS-CoV-2. Our study evaluated the performance of a Panbio™ COVID-19 test in a health care setting to guide their use in a local context. The Panbio™ COVID-19 test was commercially available in Hong Kong at the end of October 2020. In previous, study, the overall sensitivity of the Panbio kit ranged from 73.3% to 75.5% (Mak et al., 2021). This study shows that the sensitivity of the Panbio™ COVID-19 Ag Rapid Test is 82% (95% CI: 48.22 to 97.72) in symptomatic patients with a time to symptom onset ≤7 days. Among patients with a time to symptom onset ≤ 5 days, positive for SARS-CoV-2 RT-PCR, and with a Ct value ≤ 33, the sensitivity of the Panbio™ COVID-19 Ag Rapid Test is estimated to be 87.5% (95% CI:52.91 to 97.76). Some authors had previously shown that the SARS-CoV-2 virus viral load in throat or nasopharyngeal swabs peaks before the 5th day of symptom onset, and progressively decreases after this period (Wölfel et al., 2020).

Regarding specificity, it was estimated to be 97.3% among symptomatic patients, and 96.4% among RT-PCR negative asymptomatic patients. These results agree with those provided by the manufacturer and confirm that the Panbio™ COVID-19 Ag Rapid Test can be an alternative to RT-PCR in the diagnosis of COVID-19 disease in symptomatic patients with a symptom onset time of fewer than 7 days, as suggested by the manufacturer. Indeed, according to the manufacturer, the sensitivity of the Panbio™ COVID-19 Ag Rapid Test is 93.3% among symptomatic patients with a time to symptom onset of fewer than 7 days according to the user's manual. Like the sensitivity provided by the manufacturer, which is comparable to our results (confidence interval 95% CI: 52.91-97.76), the specificity of the Panbio antigen among symptomatic patients in our study also confirms that provided by the manufacturer (99.4%).

Table 2. Results of the Panbio™ COVID-19 Ag Rapid Test according to the symptomatic or asymptomatic profile of patients tested at first contact.

Panbio™ COVID-19 Ag Rapid Test results on all patients tested at the first visit			
	RT-PCR		
	Positive	**Negative**	**Total**
Panbio™ COVID-19 Ag Rapid Test Positive	21	07	28
Panbio™ COVID-19 Ag Rapid Test Negative	31	209	240
Total	52	216	268
Results of Panbio™ COVID-19 Ag Rapid Test in symptomatic patients tested at the first visit			
	RT-PCR		
	Positive	**Negative**	
Panbio™ COVID-19 Ag Rapid Test Positive	13	02	15
Panbio™ COVID-19 Ag Rapid Test Negative	12	72	84
Total	25	74	99
Panbio™ COVID-19 Ag Rapid Test results in symptomatic patients tested with a symptom onset time of 1 to 5 days			
	RT-PCR		
	Positive	**Negative**	
Panbio™ COVID-19 Ag Rapid Test Positive	7	0	7
Panbio™ COVID-19 Ag Rapid Test Negative	1	21	22
Total	8	21	29
Panbio™ COVID-19 Ag Rapid Test results in symptomatic patients tested with a symptom onset time of 1 to 7 days			
	RT-PCR		
	Positive	**Negative**	
Panbio™ COVID-19 Ag Rapid Test Positive	9	2	11
Panbio™ COVID-19 Ag Rapid Test Negative	2	34	36
Total	11	36	47
Panbio™ COVID-19 Ag Rapid Test results in symptomatic patients tested with a time to onset of symptoms greater than 7 days			
	RT-PCR		
	Positive	**Negative**	
Panbio™ COVID-19 Ag Rapid Test Positive	2	0	2
Panbio™ COVID-19 Ag Rapid Test Negative	3	4	7
Total	5	4	9
Results of Panbio™ COVID-19 Ag Rapid Test in asymptomatic patients tested at the first visit			
	RT-PCR		
	Positive	**Negative**	
Panbio™ COVID-19 Ag Rapid Test Positive	08	05	13
Panbio™ COVID-19 Ag Rapid Test Negative	19*	134	153
Total	27	139	166

*8 patients out of 19 had a Ct value<=33 (6/19 for Orf1ab and 8/19 for E gene).
Source: Authors

Our results agree other evaluations done on Panbio around the world. Indeed, during the second wave in Switzerland, for the Panbio COVID- 19 test, the clinical sensitivity was 81% and clinical specificity was 99.1%. Based on their findings, the diagnostic performance ofthe Panbio™Covid-19 test meet the criteria required by the WHO for Ag-RDTs (sensitivity ≥80% and specificity ≥97%) in a high incidence setting in symptomatic individuals (Nsoga et al., 2021).

In Spain, a multicenter evaluation of the Panbio™ COVID-19 test showed an overall sensitivity and specificity for the Panbio™ COVID-19 test were 90.5% and 98.8% respectively (Merino et al., 2021). Still in Spain, overall sensitivity was 60.0 % for the Panbio COVID- 19 test (Pérez-García et al., 2021). In the Netherlands, a prospective cohort study for SARS- CoV-2 infection in asymptomatic individuals using the Panbio COVID- 19 antigen rapid test (Abbott) compared with RT-

Table 3. Results of the Panbio™ COVID-19 Ag Rapid Test by RT-PCR Ct value and presence of symptoms.

Panbio™ COVID-19 Ag Rapid Test results by viral load (Ct value) independent of symptoms		
	RT-PCR Positive	
	Ct ≤ 33	**Ct > 33**
Panbio™ COVID-19 Ag Rapid Test Positive	14	7
Panbio™ COVID-19 Ag Rapid Test Negative	14	17
Total	28	24
Panbio™ COVID-19 Ag Rapid Test results by viral load (Ct value) in the presence of symptoms		
	RT-PCR Positive	
	Ct ≤ 33	**Ct > 33**
Panbio™ COVID-19 Ag Rapid Test Positive	9	4
Panbio™ COVID-19 Ag Rapid Test Negative	7	6
Total	16	10
Panbio™ COVID-19 Ag Rapid Test results by Ct value in patients with a symptom onset time of 1 to 7 days		
	RT-PCR Positive	
	Ct ≤ 33	**Ct > 33**
Panbio™ COVID-19 Ag Rapid Test Positive	8	1
Panbio™ COVID-19 Ag Rapid Test Negative	2	0
Total	10	1
Panbio™ COVID-19 Ag Rapid Test results by Ct value in patients with a symptom onset time of 1 to 5 days		
	RT-PCR Positive	
	Ct ≤ 33	**Ct > 33**
Panbio™ COVID-19 Ag Rapid Test Positive	7	0
Panbio™ COVID-19 Ag Rapid Test Negative	1	0
Total	8	0

Ct=Cycle Threshold.
Source: Authors

PCR, showed the sensitivity of Panbio ranged from 80.0 to 86.67% and specificity from 99.53 to 100% (Winkel et al., 2021). Also, in asymptomatic Canadians, an evaluation of the Abbott PanbioTM COVID-19 Ag rapid antigen test showed a low sensitivity (54.5%), but it allowed for faster identification of infected individuals (Shaw et al., 2021).

In contrast, the Panbio COVID- 19 test displays low sensitivity (35 to 50%) in asymptomatic close contacts of COVID-19 patients (Torres et al., 2021). Also, clinical performance of the Panbio COVID- 19 test depends on the nature of the sample. Collection of throat (sensitivity 57.7%) and saliva (sensitivity 2.6%) was stopped early due to poorer.

Nasopharyngeal swab was the best one (sensitivity 87.7%). The Panbio COVID- 19 test is suitable for patients presenting within 7 days of symptom onset using nasopharyngeal swabs. Throat and saliva swabs are not reliable specimens for the Panbio COVID- 19 test (Stokes et al., 2021). Sensitivity for samples within the first 5 days after the onset of symptoms was 91.3 % for the Panbio COVID- 19 test (Pérez-García et al., 2021). Also, (Merino et al., 2021) found in patients with threshold cycle (CT) < 25 a sensitivity was 99.5% and in participants with symptoms onset ≤5 days, it was 91.8%. Thus, the Panbio™ COVID-19 test could be easily recommended for early symptom detection (≤5 days).

In our study, the Kappa concordance between the Panbio™ COVID-19 Ag Rapid Test and RT-PCR is highly variable depending on the type of subject tested. Indeed, the best concordances between the antigenic RDT and RT-PCR are respectively observed in patients with symptoms dating from 1 to 5 days (0.91), and 01 to 07 days (0.76). This agreement is 0.37 among patients with a delay in the onset of symptoms of more than 07 days, and 0.57 among asymptomatic patients.

These results show that the use of the Panbio™ COVID-19 Ag Rapid in asymptomatic patients or beyond the first 07 days of symptom onset significantly reduces the diagnostic sensitivity of the test. Indeed, this sensitivity is 29.63% among patients with asymptomatic COVID-19 disease and 40.0% when the time to symptom onset is beyond 07 days. This seems logical especially since the SARS-CoV-2 viral load is generally at a low level among asymptomatic patients, as well as among patients with symptoms dating back more than 7 days,

Table 4. Performance of the Panbio™ COVID-19 Ag Rapid Test compared to RT-PCR.

	all patients		Symptomatic patients		Symptom onset time of 1-5 days		Symptom onset time of 1-7 days		symptom onset time more than 7 days		Asymptomatic patients	
PARAMETER	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**
Sensitivity	40.38	28.16- 53.93	52.0	33.5- 69.97	87.5	52.91- 97.76	81.82	52.3- 94.86	40.0	11.76- 76.93	29.63	15.85- 48.48
Specificity	96.76	93.46- 98.42	97.3	90.67- 99.26	100	84.54- 100	94.44	81.86- 98.46	100	51.01- 100	96.4	91.86-98.45
Positive predictive value	75.0	56.64- 87.32	86.67	62.12- 96.26	100	64.57- 100	81.82	52.3- 94.86	100	34.24- 100	61.54	35.52- 82.29
Negative predictive value	87.08	82.25- 90.75	85.71	76.67- 91.64	95.45	78.2- 99.19	94.44	81.86- 98.46	57.14	25.05- 84.18	87.58	81.42- 91.9
Accuracy of diagnosis	85.82	81.14- 89.49	85.86	77.65- 91.39	96.55	82.82- 99.39	91.49	80.07- 96.64	66.67	35.42- 87.94	85.54	79.39- 90.09
Likelihood ratio of positive test	12.46	8.206 - 18.92	19.24	6.283 - 58.92	--	--	14.73	5.266 - 41.19	--	--	8.237	3.11 - 21.81
Likelihood ratio of negative test	0.62	0.58 - 0.66	0.49	0.42 - 0.58	0.125	0.02 - 0.89	0.19	0.07 - 0.51	0.6	0.31 - 1.15	0.73	0.66 - 0.81
unweighted Cohen's kappa coefficient	0.45	0.34 - 0.56	0.57	0.38 - 0.75	0.91	0.55 - 1.27	0.76	0.48 - 1.05	0.37	-0.14 - 0.88	0.33	0.19 - 0.47

Source: Authors

Table 5. Performance of the Panbio™ COVID-19 Ag Rapid Test versus RT-PCR by Ct value.

	All patients				Symptomatic patients				Ssymptom onset time of 1-7 days				Ssymptom onset time of 1-5 days			
	Ct ≤ 33		Ct >33		Ct ≤ 33		Ct >33		Ct ≤ 33		Ct >33		Ct ≤ 33		Ct >33	
PARAMETER	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**	**%**	**95%CI**
Sensitivity	50.0	32.63-67.37	29.17	14.91-49.17	56.25	33.18-76.9	40	16.82-68.73	80	49.02-94.33	100	20.65-100	87.5	52.91-97.76	-	-
Specificity																
Positive predictive value	66.67	45.37-82.81	100	64.57-100	100	70.08-100	100	51.01-100	100	67.56-100	100	20.65-100	100	64.57-100	-	-
Negative predictive value	-		-		-		-		-		-		-		-	-
Accuracy of diagnosis	59.62	46.07-71.84	29.17	14.91-49.17	56.25	33.18-76.9	40	16.82-68.73	80	49.02-94.33	100	20.65-100	87.5	52.91-97.76	-	-

Source: Authors

taking into account the kinetics of antigens in the infected subject (Loho and Widodo, 2021; Thakur et al., 2021; Zhang and Guo, 2020). Therefore, it is not advisable to use the antigen test alone in the detection of COVID-19 disease among asymptomatic patients, especially when with a low viral load (Ct>33) or in the diagnosis of patients consulting more than 07 days after the onset of symptoms. This confirms the manufacturer's recommendation that Panbio antigen is indicated for use among symptomatic patients with less than seven days of symptom onset.

According to (Nsoga et al., 2021), a presumed cut-off for infectious virus was Ct ≤26.7 corresponding ≥ 1E6 SARS-CoV-2 genomes copies/mL. Indeed, for samples with Ct ≤ 25, sensitivity was 96.4 % for Panbio test and with Ct>25, sensitivity was 24.4 %. The Panbio COVID-19 Ag showed excellent performance and agreement results for samples with high viral loads (Ct ≤ 25) or samples taken within the first 5 days after the onset of symptoms (Pérez-García et al., 2021). Aslo, (Nordgren et al., 2021) found that The Panbio COVID-19 Ag test had high sensitivity for samples with Ct-values <25 (>88%) and no sample with a Ct-value >27 was shown to contain infectious virus with Panbio COVID-19 Ag

test.in conclusion, the Panbio COVID-19 Ag test performs well clinically, with even more reliable results for patients with a shorter clinical course of the disease or a higher viral load (Merino et al., 2021).

This study is not without its limitations, among which we could mention the low number of positive cases, with the consequence of widening the confidence intervals of the different estimated parameters. Despite these difficulties and limitations, the study was able to provide useful information for assessing the performance of the test evaluated, which could guide its use in the local context.

Conclusion

In conclusion, the Panbio™ COVID-19 Ag Rapid Test showed good performance in detecting COVID-19 cases in patients with a symptom onset time of fewer than seven (7) days. This performance is even better when this delay is reduced to fewer than 5 days. The results show that the antigenic RDT is not suitable for the detection of COVID-19 in asymptomatic patients such as travelers, or patients with a delay of more than 7 days since the onset of suspected symptoms.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Antimicrobial properties of *Moringa Stenopetala* seed oil

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Moringa stenopetala **is a multipurpose tree with considerable economic and social potential as it has vital nutritional, industrial, and medicinal applications. The study was aimed to investigate the antimicrobial activities of** ***M. stenopetala*** **seed oil against pathogenic microorganisms.** ***M. Stenopetala*** **seeds were collected from three locations (Damba Gofa, Shelle, and Konso) and extracted using two different solvents (hexane and petroleum ether). Pathogenic microorganisms: bacteria (gram-positive,** ***Staphylococcus aureus,*** **and gram-negative** ***Escherichia coli***) **and the fungal strains (*Trichophyton mentagrophytes* and *Candida albicans*) were used in this study. Standard procedures were followed to determine antimicrobial activities of** ***M. stenopetala*** **extract against pathogenic microorganisms. The result revealed that** ***M. stenopetala*** **seed extract has shown inhibitory activity against** ***T. mentagrophytes*** **fungi at the concentration ≥ 12.5% at all locations and both extraction solvents used. However, the extract did not show any inhibitory activity against tested bacteria and** ***C. albicans*** **fungi. The finding indicated that** ***M. stenopetala*** **seed could be used as an alternative to chemical fungicide to control** ***T. mentagrophytes*** **fungi. Further investigation is needed on the identification of compounds that inhibits the pathogenic microorganism.**

Key words: Antimicrobial activity, bacteria and fungi, *Moringa stenopetala* seed, extract.

INTRODUCTION

World Health Organization (WHO) reported that 80% of the population in developing countries relies on medicinal plants to acquire primary health care needs (WHO, 2002). This is likely in Ethiopia where 80% of the human population and 90% of livestock depend on traditional medicines (Abebe, 2001). The majority of these come from plant sources, which are the main sources of antimicrobial molecules (Adnan et al., 2015). These

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include secondary metabolites synthesized by the plants, more likely phenolic compounds (Hu et al., 2021). In addition, they have an advantage over synthetic products due to fewer side effects (Adnan et al., 2015).

Furthermore, they are the source of new antimicrobial drugs due to the increment of microorganisms resistant to conventional antimicrobials (Silva and Fernandes Júnior, 2010).

Moringa stenopetala belongs to the Moringaceae family and it is one of the species of the thirteenth *Moringa* geniuses (NRC, 2001). It is an underutilized, fast-growing vegetable food crop indigenous to East African lowlands and southern Ethiopia (Abuye et al., 2003). In Ethiopia, *M. Stenopetala is* commonly known as Shiferaw (Amharic), Aleko, Aluko, Halako (Gamo Gofa), Kallanki (Benishangul), Telahu (Tsemay), Haleko, Shelchada (Konso) and Haleko (Burji) (UNIDO, 2015). In English, it is named as Africa Moringa tree, Ben oil tree, Cabbage tree, and Horse-radish tree (Demeulenaere, 2001). Various parts of Moringa are used for human food, fuelwood, livestock forage, medicine, dye, water purification, soil and water conservation, quality of cooking oil, green manure, and as a source of income for Moringa cultivators (Demeulenaere, 2001; Abay et al., 2015).

M. stenopetala is used traditionally as food and to treat malaria, hypertension, asthma, diabetes, common cold, wounds, retained placenta, and stomach problems (Mekonnen and Gessesse, 1998). The seeds show a flocculating property, important in purifying turbid water (Abuye et al., 2003; Prashith et al., 2016). It is a major source of oil which could be important for cooking, salad (Raghavendra et al., 2016), and for different industrial applications (Seifu, 2015).

Furthermore, the seed possesses coagulant activity is useful for clarifying water and possesses antimicrobial activity (Rani et al., 2018). *M. stenopetala* seed extraction using different extraction solvents like hexane and methanol exhibits inhibition against waterborne disease, caused by *Salmonella typhii*, *Vibrio cholera*, and *Escherichia coli* (Walter et al., 2011). This is mainly due to biologically active compounds of a plant relying on the type of solvent used in the extraction procedure (Seleshe and Kang, 2019).

Even though *M. stenopetala* has a remarkable role in the lives of a large population of Southern Ethiopian, there is a lack of research conducted on the antimicrobial activities of *M*. *stenopetala* seed extract in the study area. Furthermore, the growing pressure on food manufacturers to avoid the use of chemical preservatives needs to search for alternative preservatives. Therefore, the present study aimed to evaluate the antimicrobial activity of *M. stenopetala* seed solvent extract collected from different locations against four pathogenic micro-organisms, namely *Staphylococcus aureus, Escherichia coli, Trichophyton mentagrophytes,* and yeast *Candida albicans.*

MATERIALS AND METHODS

Samples collection

The identification of *M. stenopetala* used in this study was done with the help of a botanist from Arbaminch university and the dominance of *M. stenopetala* in the sites considered by the study (Abuye et al., 2003; Gebregiorgis et al., 2012; Seifu, 2015). Matured pods of *M. stenopetala* with similar color were collected from three locations in Southern Ethiopia; Gofa Zone (Demba Gofa district), Gamo Zone (Shelle district), and Segen Area Zone (Konso district) from January to February 2022. The locations were selected purposely based on the availability and abundance of *M. stenopetala* trees in the area.

Shelle district is located about 27 km from Arba Minch town and 532 km from Addis Ababa. Demba Gofa district is located 526 km from Addis Ababa. Konso district is located about 600 km southwest of Addis Ababa capital city of Ethiopia.

M. stenopetala seed powder preparation

The powder preparation was performed following the procedure indicated by Haile et al. (2019). Briefly, the matured seeds were separated from their pods and cleaned by removing the bark. The seeds with even appearance in size and shape were selected. The seeds were sun-dried to separate the husk from the seed kernel and the seed powder was prepared using a mechanical grinder. The powders obtained were sieved and then stored in polythene bags until extraction at Arba Minch University Chemistry laboratory.

Oil extracts preparation

The oil was extracted using a semi-continuous process; soxhlet procedure, through repeated washing (percolation) with n-Hexane and petroleum ether. Seed powders of 40 g were placed in a porous cellulose thimble. Then the timble was placed in an extraction chamber in between flask containing solvents of 150 ml and condenser. Heat was applied into the flask where the solvent evaporates into a condenser and converted to liquid that flows into the extraction chamber containing the sample. At the end of extraction, the remaining solvent in a flask is evaporated in an oven and the oil was collected (Adejumo et al., 2013).

Test organisms

The pathogenic microorganisms used in this study were gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*; the fungal strains *T. mentagrophytes* and *C. albicans* (Yeast). The strains were clinical isolates obtained from Bacteriology and Mycotic disease reference laboratory of Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

Inoculum preparation

The inoculum for bacteria was prepared from the stock cultures and sub cultured onto nutrient agar using a sterilized wire loop and incubated at 37^{o}C for 24 h. Whereas the yeast and fungi were inoculated with Sabouraund Dextrose Agar (SDA) media and

incubated at 25^{o}C for 72 h. The required working suspension of the inoculum was prepared by transferring morphologically similar colonies of each organism from a young culture in 5 ml nutrient broth (for bacteria) and Sabouraud Dextrose Broth (SDB) for fungi. Then the turbidity of the inoculum was standardized to 0.5 McFarland turbidity standards by measuring with OD 600 nm spectrophotometer to have inoculum size which is equivalent to 1x106^{-8} CFU/ml. Then the suspension was diluted to 1:100 and used as a starting inoculum for the test (Cheesbrough, 2002).

Controls used in the study

Chloramphenicol for *S. aureus* and *E. coli* and Ketoconazole for *T. mentagrophytes* and *C.albicans* was used as a positive control but 5 % Tween 80 was utilized as a negative control.

Antimicrobial assay

Antibacterial activity of n-Hexane and petroleum ether extracts of *M. stenopetala* seed oil were evaluated by the modified agar well diffusion technique (Bauer et al.,1996). Standardized inoculum of bacterial and fungal culture suspension was uniformly swabbed on the Mueller Hinton Agar (MHA) (OXOID) and SDA (PARK) media respectively by using a sterile cotton swab. The inoculated plates were left at room temperature for 10 minutes to absorb any surface moisture before applying the extract. Thus, wells were aseptically punched on both MHA and SDA plates equidistant of 6 mm in diameter by using a sterile stainless still borer and labeled at the backside of the plates. Each well was filled with 100 µl of n-hexane and petroleum ether extracts at concentrations of 3.13, 6.25, 12.5, 25 and 50%. Accordingly, all plates were kept to settle down on a working bench for 1hr to allow proper diffusion of the extract into the media. The bacteria cultures were incubated at 37ºC for 24 h while the fungal culture was incubated at 25ºC for 72 h. The solvents that were used to reconstitute the extract were set up in parallel. Antimicrobial activity was determined by measuring the zone of inhibition around each well. For each extract duplicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the seed oil was determined against the test organisms by using the agar dilution technique (Griffin et al., 2000). This was conducted by mixing the sterile cooled at 45°C MHA and SDA media with different concentrations (4 and 2%) of n-Hexane and petroleum ether extract and poured into Petri dishes (90 mm) and left to solidify then the plates were left upside down at room temperature for 10 to 15 minutes to avoid moisture. In the same fashion controls without the extract were set up in parallel using 5% Tween 80 for negative control and Chloramphenicol and Ketoconazole for positive control. Mueller Hinton Agar and SDA were inoculated with the strains to confirm the viability of the culture. Followed by these 10 µl from each standardized bacterial and fungal suspension was taken and inoculated on the media that were incorporated with plant extracts. The plates were allowed to stand for 5 min and incubated at 37ºC for 24 h for bacteria and 25ºC for 72 h for fungi. The procedure was performed in duplicate at different concentrations of the extract.

Statistical analysis

The zone of inhibition around each disc was measured in mm and the results were presented as means ±SD using IBM SPSS Statistics software (version 25).

RESULTS

Antibacterial activity of *M. stenopetala* seed oil

The results have shown that n-Hexane and petroleum ether extracts of *M. stenopetala* seed from the different locations used at different concentrations has shown no zones of inhibition of bacterial growth (Table 1). The inhibition zone for the standard drug chloramphenicol was 11.8 mm for *E. coli* and 14.0 mm for *S.aureus*.

Antifungal activity of *M. stenopetala* seed oil

Minimum inhibitory concentration of M. stenopetala seed oil

The finding of this research has indicated no inhibition for all tested microorganisms at the concentrations of 2 and 4%. Furthermore, no inhibitory effect was observed in the presence of 5% Tween 80 which was used as a negative control (Table 1).

DISCUSSION

The study did not show any inhibition activity of *M. stenopetala* extracts against bacteria. Previous study reported controversial results from the present study (Chekesa and Mekonnen, 2015); methanol crude extract and ethyl acetate extract of the *M. stenopetala* seeds showed the highest antibacterial activity, against *S. aureus* and *E. coli* but petroleum ether extract of the seeds only showed inhibition on *S. aureus* but not in *E.coli*. The resistance of *E. coli* to the extract matches findings from a study on the antibacterial activity of *Moringa* leaf extract to be ineffective against *E.coli (*Bhawasar et al., 1965; Peixoto *et al*., 2011). In line with the current study petroleum ether leaf extract of *M.olifera* didn't show inhibition against *S.aureus* and *E.coli* isolated from urinary tract-infected patients (Abdalla et al., 2016).

The organisms that are included in this study are clinical isolates that are obtained from symptomatic patients. Hence, they may have a high chance of exposure to anti-bacterial agents that may bring change to the molecular and other factors. Therefore, the microorganisms are expected to be less sensitive compared to standard organisms with no chance of exposure to any antimicrobial agents. Moreover, a previous study (Rahman et al., 2008) reported that petroleum ether extract from the stem bark of *M. oleifera* did not show antibacterial activity in both *E. coli and S.*

Table 1. Antifungal effect of different solvent extracts of *M. stenopetala* seed oil.

Zone of inhibition (mm)				
Test organisms				
Location	**Extraction solvent**	**Concentration (%)**	***T. mentagrophytes* (mean±SD)**	***C. albicans***
Shelle	Pet ether	50	14±0.28	-
		25	10±0.28	-
		12.5	7±0.25	-
		6.25	-	-
		3.13	-	-
Shelle	n-Hexane	50	17.75±0.73	-
		25	17±0.32	-
		12.5	12.38±0.01	-
		6.25	11.2±0.01	-
		3.13	11±0.63	-
Goffa	Pet ether	50	12±0.91	-
		25	7±0.21	-
		12.5	-	-
		6.25	-	-
		3.13	-	-
Goffa	n-Hexane	50	10±0.28	-
		25	7.5±0.19	-
		12.5	5±0.28	-
		6.25	-	-
		3.13	-	-
Konso	Pet ether	50	10±0.14	-
		25	8±0.50	-
		12.5	7±0.77	-
		6.25	-	-
		3.13	-	-
Konso	n-Hexane	50	9±0.35	-
		25	7±0.14	-
		12.5	-	-
		6.25	-	-
		3.13	-	-
Ketokonazo		0. 1 mg/ml	21.0±0.05	15.4±0.00
Dist. H_2O Negative control Tween80		5%	-	-

Mean±SD- mean±standard deviation, _ No inhibition zone (no activity), Pet ether-Petroleum ether.
Source: Authors

aureus. Furthermore, a study made by Shailemo et al. (2016) showed antimicrobial activity *M. oleifera* n-Hexane seeds and bark extracts against pathogens of water-borne diseases was lower than other solvents used for extraction. The inactivity of both extracts against bacteria might be because of the presence of polar compounds in the plant that can bind to the cytoplasmic membrane of the organism but since both the extracts are non-polar the activity of the compound becomes inactive against the tested organism (Boyd and Beveridge, 1981).

Both n-Hexane and petroleum ether extract of *M. stenopetala* seed showed antifungal activity against *T. mentagrophytes* at the concentration ≥ 12.5% except n-Hexane extract collected from Shelle which has shown antifungal activity at the concentration of ≥ 3.06. In line with the current finding, Dinesha et al. (2018) reported that Moringa seed kernel oil presented excellent antifungal activities. Furthermore, Anthonia (2012) reported that *T. mentagrophyte* growth was inhibited by inhibition zone of 22 mm using ethanolic extract *M. oleifera* leave. In other study, *M. stenopetala* methanolic leaf extract results in concentration dependent inhibition of mycelial growth of *Aspergillus flavus* (Kekuda et al., 2016).

The result has demonstrated an increase in the exteraction concentration resulted in gradual increases in the inhibition zone. Similar result has been reported by Prabakaran et al. (2018) for *M. oleifera* extract. Both n-Hexane and petroleum ether extract of *M. stenopetalla* seed has shown no antifungal activity against *C. albicans* (Table 1). This result was in line with a study conducted by Rahman et al. (2008) where petroleum ether extract from the stem bark of *M. oleifera* did not show antifungal activity against *C. albicans.* The inhibition zone for the standard drug Ketoconazole was 21.0mm for *T. mentagraphyte* and 15.4 mm for *C. albican.* In a study done by Lalas et al. (2012) *Moringa peregrina* seed oil extracted by n-Hexane a low activity to *C. albicans* was found compared to other microorganisms *C. albicans* was also found to be the most resistant compared to the tested organism for cold pressed and n-Hexane extracted *Moringa peregrina* seed oil (Osman et al., 2022). In our study both n-Hexane and petroleum ether extract did not show any activity against *C. albicans* this might be due to different species of Moringa.

Generally, the variations in the antimicrobial activities of different study reports could be due to differences in Moringa species, environment conditions, extraction methods, extraction solvent used, age and parts of Moringa used.

Conclusion

The results of the study revealed that *M. stenopetala* seed extract has shown the potential to inhibit the activities of *T. mentagraphyte* fungi even at a lower concentration. The result of the present study is promising as the *M*. *stenopetala* seed extract exhibited marked antifungal potential which could be used as an alternative to the fungicide chemical. Further studies need to be conducted with various pathogenic micro-organisms and extraction with more polar extraction solvents such as Carbon tetrachloride, chloroform, ethyl acetate, etc. Identification of compounds that are responsible to inhibit pathogenic microorganisms also needs further investigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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